FEB 14 1945

Official Organ of the Society for the Study of Development and Growth

GROWTH

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A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

VOLUME VIII, NUMBER 4
PAPERS 265 - 270

DECEMBER, 1944 $5.00 A YEAR

Entered as second class matter on October 20, 1941, at the Post Office, Worcester, Mass., Under the Act of March 3, 1879.

GROWTH

A journal for the coördination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a coöperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy, and anthropology; just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines, and hormones.

The journal will appear regularly at quarterly intervals. Subscription is $5.00 per volume (outside U. S. and Canada, $5.50). Single numbers $2.00. There will be one volume of at least 400 pages each year.

Subscriptions, manuscripts submitted for publication, and all correspondence should be sent to *The Editors of Growth, Lankenau Hospital Research Institute, Girard and Corinthian Avenues, Philadelphia 30, Pa.*

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Growth, 1944, **8**, 235-249.

APPLICATION OF THE THEORY OF ABSOLUTE RATES TO BIOLOGICAL PROCESSES

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A. INTRODUCTION

In the course of discussions with Dr. A. R. Moore on the subject of the influence of temperature on biological processes, such as regeneration, egg division, etc., I had my attention called to the very unsatisfactory results obtained by biologists in the use of the Arrhenius equation (1889). An adequate picture of the present confused situation in this field is available, for instance, in Heilbrunn's *Outline of General Physiology* (1943), where references to the early work on the subject, as well as a discussion of the more recent work of Crozier and collaborators (1926), will be found in the section entitled "Temperature and Rate of Biological Processes." If Heilbrunn's discussion can be regarded as representative of the present use of the theory of chemical kinetics in biological kinetics, one can only conclude that the fundamental developments of the last few years in the theory of rate processes have not yet exerted any influence on the interpretation of biological rate data. Indeed, there is no difference between Heilbrunn's exposition of the use of the Arrhenius equation and that of Kanitz (1915). The considerable advances in the field of chemical kinetics since the days of Arrhenius are now available in a detailed and organized form in the book of Glasstone, Laidler, and Eyring (1941). The evolution of the original Arrhenius treatment into Eyring's present theory of absolute rates (1935, 1938) has been a gradual one. Some fundamental changes, such as the replacement of the Arrhenius energy of activation by free energy and the introduction of the concept of entropy of activation, were suggested as early as 1911 by Kohnstamm and Scheffer. Another important new idea, particularly useful in the case of reactions in solutions, that of the catalytic factor, was introduced by Bronsted in (1922). A few reactions involving biological materials

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(but in a non-living condition) have been examined in the light of the Eyring theory: the inactivation of enzymes by heat, studied by Sizer (1943) and the denaturation of proteins, studied by Stearn and Eyring (1937).

Even a casual inspection of biological rate data shows that the use of the Arrhenius equation is unjustified. Not only do the Q_{10} ratios between specific rates corresponding to temperatures 10 degrees apart vary too rapidly with temperature to be consistent with the Arrhenius concept, but the energies of activation (also called "critical thermal increments" or "temperature characteristics") likewise vary considerably with temperature, in direct conflict with the fundamental assumption of the theory that μ be constant. The interpretation of the rates of biological reactions requires the use of the new theory of Eyring and others more imperatively even than ordinary chemical reactions in non-living matter, where the Q_{10} ratios and the μ's vary much less with temperature than in the case of the biological reactions. In the next section of this paper I present a summary of the Arrhenius theory and of its unsuccessful applications to biological reactions.

B. The Arrhenius Theory of Activation and Its Failure with Biological Reactions

In the Arrhenius theory the specific reaction rate k is given by the expression

$$k = Ae^{-\frac{\mu}{RT}} \tag{1}$$

in which A is independent of, or varies little with, temperature, e is the base of natural logarithms, μ is the energy of activation or critical thermal increment, R is the gas constant, T the absolute temperature. When μ is expressed in calories R is equal to 2. Applying (1) to two absolute temperatures T_1 and T_2 we have

$$k_2 = k_1 e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \tag{2}$$

The Q_{10} ratio is the ratio k_2/k_1 when the temperature T_1 and T_2 are 10 degrees apart:

$$Q_{10} = \frac{k_{T+10}}{k_T} \tag{3}$$

It can be obtained from the rates at any two temperatures T_1 and T_2 by the formula

$$Q_{10} = \left(\frac{k_1}{k_2} \right)^{\frac{10}{T_1 - T_2}} \tag{4}$$

This Q_{10} ratio should be regarded as corresponding to the temperature half-way between T_1 and T_2, i.e, $\frac{1}{2}(T_1 + T_2)$, and the 10° interval is thus from $\frac{1}{2}(T_1 + T_2) - 5$ to $\frac{1}{2}(T_1 + T_2) + 5$. It is clear that the interval $T_1 - T_2$ should not be too different from 10°. Taking the natural logarithm of k from (1) we get

$$ln\ k = ln\ A - \frac{\mu}{RT} \tag{5}$$

and, leaving μ and A constant,

$$\frac{d\ ln\ k}{dT} = \frac{\mu}{RT^2} \tag{6}$$

An interpretation of experimental values of k by means of the Arrhenius theory could be satisfactory only if a plot of $ln\ k$ against $1/T$ turns out to be linear. Another way of testing the theory is to calculate μ for the different values of Q_{10} by means of the formula

$$\mu = 0.46\ T(T + 10) log_{10}\ Q_{10} \tag{7}$$

which can easily be deduced from (5). Taking as typical example A. R. Moore's data on regeneration in Tubularia crocea (1910) we find the values of μ reported in Table 1. The variation of μ is con-

TABLE 1

VALUES OF μ FOR DIFFERENT 10° INTERVALS: REGENERATION IN TUBULARIA CROCEA

Temperature interval	Q_{10} Experimental	μ Calories	Q_{10} Calculated for μ = 22,700 cal.
6°-16°	4.1	22,700	4.10
8°-18°	4.0	22,600	4.02
10°-20°	3.6	21,200	3.94
12°-22°	2.8	17,300	3.86
13°-23°	2.7	16,800	3.82

siderable for such a small range of temperature. For comparison we give in Table 2 data covering a similar range of temperature for the ordinary chemical reaction $C_2H_5I + C_2H_5ONa \rightarrow C_2H_5\text{-}O\text{-}C_2H_5 + NaI$. This table is based upon a similar one given by Kanitz. In this case the Q_{10} ratios are practically constant and μ increases slowly. Kanitz gives several reactions for which Q_{10} varies just enough to

TABLE 2
VALUES OF μ FOR DIFFERENT 10° INTERVALS: FORMATION OF DIETHYL ETHER

Temperature interval	Q_{10}	μ calories
1°-11°	3.32	18,600
10°-20°	3.26	19,600
16°-26°	3.40	21,200
22°-32°	3.38	21,800

make μ practically constant. The last column of Table 1 gives the Q_{10} ratios which would correspond to μ constant and equal to the value 22,700 cal. calculated for the 6°-16° interval. It is clear that the Arrhenius equation does not apply to this process, and this is characteristic of the general situation with biological reactions.

Crozier's use of the Arrhenius equation in the identification of "master reactions" holding sway between definite intervals of temperature can hardly be regarded as a solution of the difficulties. Examination of the abundant experimental material used by Crozier shows that the variation of μ with temperature is, within the limits of experimental error, continuous and it follows therefore that the Arrhenius equation is not applicable.

It is, moreover, possible to show that taking into account a variation of μ with temperature corresponding to

$$\mu_2 = \mu_1 + C(T_2 - T_1) \tag{8}$$

in which C would represent the heat capacity change corresponding to activation, would not remove the difficulty. Formula (2) would become

$$k_2 = k_1 e^{\frac{1}{2}\left(\frac{\mu_1}{T_1} - \frac{\mu_2}{T_2}\right)} \tag{9}$$

For a 10° interval $T_1 - T_1 + 10$ one would obtain

$$\mu_1 - CT_1 = 0.46\, T_1(T_1 + 10)\, log_{10}\, Q_{10,\, T_1} \tag{10}$$

a formula which would replace (7). Writing it for another 10° interval $T_2 - T_2 + 10$, we have

$$\mu_2 - CT_2 = 0.46\, T_2(T_2 + 10)\, log_{10}\, Q_{10,\, T_2} \tag{11}$$

From (8) one sees that

$$\mu_1 - CT_1 = \mu_2 - CT_2 \tag{12}$$

and hence that the expressions on the right-hand sides of (10) and (11) should be equal. These, however, are the μ's calculated previ-

ously on the assumption that μ does not vary with temperature and we found, in the case of A. R. Moore's data, that these μ's do not turn out to be constant. Before giving up the Arrhenius equation one might inquire into the possibility that C, the heat capacity change upon activation, could be a function of temperature. This does not lead to anything of interest. The difficulties with the Arrhenius equation are of a much deeper nature and are all due to the fact that the free energy of activation should be used in place of the energy of activation.

C. The Eyring Equation for the Absolute Specific Rate of a Chemical Reaction—Its Use in the Interpretation of the Q_{10} Ratios of Biological Processes

The specific rate k of a chemical reaction has been shown by Eyring and collaborators to be of the following form:

$$k = \frac{KT}{h} e^{-\frac{F}{RT}} \tag{13}$$

in which K is the molecular gas constant (R divided by Avogadro's number), h is Planck's constant or quantum of action, and F is the free energy of activation. For a reaction in which AB is the reaction complex, $A + B \rightarrow AB \rightarrow C + D$, F is the standard free energy difference:

$$F = \Delta F^{\circ} = F_{AB}{}^{\circ} - (F_A{}^{\circ} + F_B{}^{\circ}) \tag{14}$$

In formula (13) we are neglecting a coefficient of the order of unity called the "transmission coefficient." Differentiating $ln\ k$ with respect to T we now get, instead of (6),

$$\frac{\partial ln k}{\partial T} = \frac{1}{T} + \frac{H}{RT^2} \tag{15}$$

in which H is the change of heat content upon activation. Introducing the entropy change upon activation, S, we have

$$F = H - TS \tag{16}$$

Useful thermodynamic formulas to remember are

$$\frac{\partial \frac{F}{T}}{\partial T} = -\frac{H}{T^2} \tag{17}$$

upon which (15) is based, and

$$\frac{\partial F}{\partial T} = -S \tag{18}$$

as well as Kirchoff's formula

$$\frac{\partial H}{\partial T} = C, \tag{19}$$

C being the heat capacity change upon activation already introduced above. We have, moreover,

$$\frac{\partial S}{\partial T} = \frac{C}{T} \tag{20}$$

In all these differential formulas it is understood that pressure is constant.

The Q_{10} ratio now becomes, as can easily be derived from (13),

$$Q_{10} = \frac{k_{T+10}}{k_T} = \frac{T+10}{T} e^{\frac{1}{R}\left(\frac{F_T}{T} - \frac{F_{T+10}}{T+10}\right)} \tag{21}$$

or, on the basis of (16),

$$Q_{10} = \frac{T+10}{T} e^{\frac{1}{R}\left(\frac{H_T}{T} - \frac{H_{T+10}}{T+10}\right)} e^{\frac{1}{R}(S_{T+10} - S_T)} \tag{22}$$

Taking the natural logarithm we have

$$ln Q_{10} = ln\left(1 + \frac{10}{T}\right) + \frac{H_T(T+10) - H_{T+10}T}{RT(T+10)} + \frac{S_{T+10} - S_T}{R} \tag{23}$$

Integration of (19) gives

$$H_{T+10} = H_T + \int_T^{T+10} C dT \tag{24}$$

In the present discussion we shall consider C as independent of temperature. Therefore:

$$H_{T+10} = H_T + 10C \tag{25}$$

and

$$H_T(T+10) - H_{T+10}T = 10(H_T - CT) \tag{26}$$

Integration of (20) gives

$$S_{T+10} = S_T + \int_T^{T+10} \frac{C}{T} dT \tag{27}$$

Assuming again that C is independent of temperature we find

$$S_{T+10} = S_T + C ln\left(1 + \frac{10}{T}\right) \tag{28}$$

Formula (23) thus becomes

$$ln Q_{10} = ln\left(1 + \frac{10}{T}\right) + \frac{10(H_T - CT)}{RT(T+10)} + \frac{C}{R} ln\left(1 + \frac{10}{T}\right) \tag{29}$$

For convenience in the calculations we shall make use of the following approximation, which is entirely adequate for our purpose in view of the usual degree of accuracy of biological rate data,

$$ln\left(1 + \frac{10}{T}\right) = \frac{10}{T} \tag{30}$$

This enables us to rewrite (29) as follows:

$$\frac{R}{10} ln Q_{10} = \frac{H_T - TC}{T(T+10)} + \frac{C+R}{T} \tag{31}$$

or, changing to common logarithms and replacing R by 2,

$$0.46T(T+10) log_{10} Q_{10} = H_T + 10C + 2T + 20 \tag{32}$$

a formula which replaces formula (7) based on the Arrhenius theory:

$$0.46T(T+10) log_{10} Q_{10} = \mu \tag{33}$$

Setting up Equation (32) for two different 10-degree intervals $T_1 - T_1 + 10$ and $T_2 - T_2 + 10$, and taking into account that

$$H_{T_2} = H_{T_1} + C(T_2 - T_1) \tag{34}$$

we get the two simultaneous equations:

$$\begin{gathered} 0.46T_1(T_1+10) log_{10} Q_{10,T_1} = H_{T_1} + 10C + 2T_1 + 20 \\ 0.46T_2(T_2+10) log_{10} Q_{10,T_2} = \\ H_{T_1} + [10 + (T_2 - T_1)]C + 2T_2 + 20 \end{gathered} \tag{35}$$

in which we have two unknowns: H_{T_1} and C.

Let us take, for instance, A. R. Moore's data, already treated above by the Arrhenius theory. For the 6°-16° interval we have:

$$22{,}700 = H_{6°} + 10C = 578 \tag{36}$$

and for the 13°-23° interval:

$$16{,}800 = H_{6°} + 17C = 592 \tag{36'}$$

We see that differences between such numbers as 592 and 578 will

be negligible compared with differences between μ values. Let us note, in passing, that the μ values previously calculated will still be useful as giving the left-hand sides of formulas of the type (35). From equations (36) and (36′) we deduce $C = -850$ calories per degree. Repeating these calculations for the five Q_{10} ratios of Table 1 taken 2 by 2 we obtain 10 values of C whose average is practically $C = -1{,}000$ calories per degree. It is clear that experimental errors are greatly magnified in these determinations of C. Nevertheless the values found for this case are all negative and do not follow any definite trend with temperature, which justifies our procedure of taking the arithmetic average and of neglecting the variation of C with temperature in the theory. In round figures the values of C are those given in Table 3, in which the five Q_{10} ratios are numbered from 1 to 5:

TABLE 3

VALUES OF C FOR DIFFERENT PAIRS OF Q_{10} RATIOS: REGENERATION IN TUBULARIA CROCEA

	2	3	4	5
1	—50	—380	— 910	— 850
2		—720	—1340	—1170
3			—1960	—1440
4				— 500

Adopting this value $C = -1{,}000$, we introduce it into each of the five equations of the type (36) and obtain 5 values of H_6, as follows:

6°-16°	:	32,000 calories
8°-18°	:	33,800 calories
10°-20°	:	34,300 calories
12°-22°	:	32,200 calories
13°-23°	:	32,600 calories

The average of these 5 values is 33,000 calories. At any other temperature we have:

$$H_t = 33{,}000 - 1{,}000\ (t - 6) \qquad (37)$$

At the highest temperature considered in the measurements, 23°, we have $H_{23} = 16{,}000$ calories. The amount of energy necessary for activation thus drops at the rate of 1,000 calories for each one degree of temperature. These H values correspond to the formation of some average mole of reaction complex from the reactants. Several reactions are no doubt occurring simultaneously in this regeneration

process, each one with its own mechanism, and the thermodynamic quantities we are dealing with here are composite values for all these simultaneous reactions. With the values of H_t given by (37) and the constant value $C = -1{,}000$ cal., we can calculate by means of (35) the Q_{10} ratios corresponding to the different 10° intervals of Table 1. We find the results given in Table 4. Since in the Q_{10} ratios only

TABLE 4

MEASURED AND CALCULATED Q_{10} RATIOS FOR THE REGENERATION IN TUBULARIA CROCEA

Temperature interval	Q_{10} experimental	Q_{10} calculated	Difference exp.-calc.
6°-16°	4.1	4.3	−0.2
8°-18°	4.0	3.8	+0.2
10°-20°	3.6	3.3	+0.3
12°-22°	2.8	2.9	+0.1
13°-23°	2.7	2.7	0.0

differences of entropies appear we cannot obtain entropies of activation from these ratios. However, by the use of the absolute rates as given by the Eyring formula (13), these absolute entropies can be obtained. This will be done in the next section of this paper. We have, for the case here studied,

$$S_{T_2} = S_{T_1} - 1{,}000\, ln\, \frac{T_2}{T_1} \tag{38}$$

or, if the interval $T_1 - T_2$ is small enough,

$$S_{T_2} = S_{T_1} - 1{,}000\, \frac{T_2 - T_1}{T_1} \tag{39}$$

Referring all these entropies to the one for 8° (lowest temperature for which an absolute rate was measured) we have

$$S_t = S_8 - 3.6\ (t - 8) \tag{40}$$

The significance of this decrease of entropy of activation resulting from an increase of temperature will be discussed later.

D. CALCULATION OF ENTROPIES OF ACTIVATION IN BIOLOGICAL PROCESSES

Formula (13) can be rewritten, on account of (16), as

$$k = \frac{KT}{h}\, e^{-\frac{H}{RT} + \frac{S}{R}} \tag{41}$$

For any particular temperature we can obtain H, the heat of activation, by the method explained in the previous section of this paper. It follows then that S, the entropy of activation, can be deduced from the absolute rate. We have

$$S = \frac{H}{T} + R\ln \frac{h}{KT} + R\ln k \tag{42}$$

With $K = 1.37 \times 10^{-16}$ and $h = 6.55 \times 10^{-27}$ we find, at 8° for instance, $KT/h = 5.88 \times 10^{12}$. From (37) we have $H_8 = 31{,}000$ and thus,

$$S_8 = 51.4 + R\ln k_8 \tag{43}$$

The problem is now to determine the true absolute k from the experimental data. The complete expression for the rate of growth, regarded as a chemical rate, is of the form

$$-\frac{dC}{dt} = kC \tag{44}$$

in which C is some average molar concentration of the molecules used in the growth process. For a finite change of concentration ΔC we would have

$$-\frac{\Delta C}{\Delta t} = kC \tag{45}$$

or

$$k = \frac{1}{\Delta t}\left(-\frac{\Delta C}{C}\right) \tag{46}$$

At the particular time when $-\Delta C = C$, i.e., when as much of the new material resulting from growth has been formed as there is material yet to be used for growth, we have

$$k = \frac{1}{\Delta t} \tag{47}$$

As it is unlikely that the specific rate would vary much during the whole growth process, it seems reasonable to take for k the value of Δt corresponding to one-half of the total time of growth or regeneration. No large change would result in the entropy values if any other end-point were chosen. The difference between $S_{1/2}$ calculated for the time of total growth would be

$$S_{1/2} - S_1 = R\ln 2 = 1.38 \tag{48}$$

a quantity small compared with $S_{\frac{1}{2}}$ or S_1 themselves. For the same reason the neglect of the transmission coefficient in the Eyring formula (41) is without importance, for our purpose, in the determination of entropies of activation, as it is unlikely that this transmission coefficient be of an order of magnitude sufficiently different from unity to affect the entropy values appreciably. Even with a transmission coefficient of 10 or one of 0.1 the entropy would differ from that calculated with a transmission coefficient of one by $R\ln 10$ or 4.6.

In this manner, using the half-time mark and a transmission coefficient of one, we find, from (43), $S_8 = 27.8$ calories per mole per degree. At any other temperature we have, using the approximation (30),

$$S_t = S_8 - \frac{1{,}000(t-8)}{281} = 27.8 - 3.54(t-8) \qquad (49)$$

At the highest temperature considered in A. R. Moore's measurements, 23° C., we have $S_{23} = -25.7$. In other words the entropy of activation changes sign somewhere between 8° and 23°, actually at 15.9° C. It is to be noted that a direct calculation of S_{23}, from the measured rate for that temperature, gives the same result as that obtained from (49).

Summarizing this case we have:

$$H_t = 33{,}000 - 1{,}000(t-6)$$
$$S_t = \quad 27.8 - 3.54(t-8)$$

The free energy at 8° is

$$F_8 = H_8 - 281S_8 = 23{,}200 \text{ cal.}$$

At 23°:

$$F_{23} = H_{23} - 296S_{23} = 25{,}600 \text{ cal.}$$

We see that the free energy varies little in this temperature interval, which means that the equilibrium constant between the complex and the reactant is little affected by temperature. We have, in general:

$$F_t = 33{,}000 - 1{,}000(t-6) - (273+t)[27.8 - 3.54(t-8)] \qquad (50)$$

or

$$F_t = 23{,}700 - 91t + 3.54t^2 \qquad (51)$$

It is interesting to note that F_t cannot vanish for any real temperature, the roots of the equation obtained by setting $F_t = 0$ in (51)

being imaginary. On the other hand H_t vanishes for $t = 39°$ C. We found that $S_t = 0$ for $t = 15.9°$.

Similar calculations carried out with the data on the rate of segmentation of sea urchin eggs of Loeb and Chamberlain (Dr. M. M. Moore) (1915) gave the following results:

$H_8 = 41{,}700$ cal., $C = -1{,}600$ cal., and hence

$$H_t = 41{,}700 - 1{,}600(t - 8) \tag{52}$$

The entropy of activation was calculated for 10° and found to be $S_{10} = 51.6$. Hence

$$S_t = 51.6 - \frac{1{,}600(t - 10)}{283} = 51.6 - 5.65(t - 10) \tag{53}$$

We see that H_t is zero for $t = 34°$ and S_t is zero for $t = 19.5°$. Again we found that the free energy F_t varies little with temperature: $F_{10} = 23{,}850$ cal. and $F_{25} = 24{,}400$ cal. With the values of H_t given by (52) and the constant value $C = -1{,}600$ cal. we can calculate by means of (35) the Q_{10} ratios corresponding to the different 10° intervals. We find the results given in Table 5.

TABLE 5

SEGMENTATION OF SEA URCHIN EGGS

Temperature interval	Q_{10} experimental	Q_{10} calculated	Difference exp.-calc.
8°-18°	6.0	5.0	+1.0
9°-19°	4.5	4.5	0.0
10°-20°	3.7	4.0	—0.3
11°-21°	3.3	3.6	—0.3
12°-22°	3.2	3.3	—0.1
13°-23°	2.8	3.0	—0.2
14°-24°	2.8	2.7	+0.1
15°-25°	2.5	2.4	+0.1

E. CONCLUSIONS

The foregoing theoretical considerations and their application to two typical examples show that the old Arrhenius theory of reaction velocities, with its concept of an energy of activation independent of temperature, is even less tenable in the case of biological processes than in ordinary chemical reactions in non-living systems. The Eyring theory of absolute rates, on the other hand, gives a logical and, we may say, "biologically promising" picture. If the reaction complex and its various properties (thermodynamic equilibrium with the

reactants, universal frequency of unimolecular decomposition KT/h, rôle played by the so-called free energy of activation, etc.) provide the correct treatment of the velocities of biological processes, a number of important new ideas of significance for biological theory can be developed. We shall restrict ourselves to a brief enumeration of these ideas, with the hope that they will be taken up and developed by biologists.

The rather rapid variation with temperature of both the energy and the entropy of activation (with the relative constancy of the free energy of activation) indicates that, as temperature rises, the height of the energy hill which the complex has to climb decreases, and the "looseness" of the complex (of which the entropy of activation is the exact measure) also decreases. The amount of "looseness" is the larger the greater the number of degrees of freedom of the complex through which the energy of activation has to be channeled in order to lead to the transformation of the complex into the products of the reaction. The relative constancy with temperature of the free energy of activation implies that, for given concentration of reactants, the concentration of the complex stays nearly constant, although, as explained below, there is actually a minimum in this concentration. In both examples considered above the entropy of activation becomes negative at a certain temperature. This means that, above this temperature, the complex is less "loose" or is "stiffer" than a simple aggregate of the reactants. The higher temperature makes it possible for the complex to change into the products by channeling the energy of activation through fewer degrees of freedom than at lower temperatures. It does not seem impossible that the temperature at which the entropy of activation is zero could be one of optimum functioning for the process in question. The entropy of activation is connected with the free energy of activation by the relation

$$\frac{\partial F}{\partial T} = -S \tag{54}$$

and the second derivative of F is

$$\frac{\partial^2 F}{\partial T^2} = -\frac{\partial S}{\partial T} = -\frac{C}{T} \tag{55}$$

C being negative in the examples above we see that, when $S = 0$,

F is a maximum. The equilibrium constant between the complex and the reactants,

$$K = e^{-\frac{F}{RT}} \tag{56}$$

is then a minimum. Hence the process takes place with fewer complexes than at any other temperature, for given concentrations of the reactants. In addition, the complex has exactly the same amount of "looseness" as any aggregate of the reactants. Whether such conditions are really optimum ones for the organism we shall leave to the biologist to speculate upon and decide. We are merely offering here the main lines of a new method of treatment and interpretation of the rates of biological processes from the point of view of the physical chemist. The fact that it is generally recognized that the Arrhenius theory has failed in biology, and the many satisfactory features of the present application of the Eyring theory of absolute rates will perhaps induce some biologists to go further into this subject, to which a physical chemist cannot contribute more than a general introduction.

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THE EFFECTS OF EXTRACTS OF ADULT, EMBRYO, AND TUMOR TISSUES ON THE GROWTH OF YEAST

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It has often been assumed that rapidly proliferating tissues, such as those of the embryo, should be richer in growth substances than adult tissues. For this reason it is customary in tissue culture work to employ embryonic extracts in spite of the fact that Carrel (3), who was responsible for the use of such extracts, showed many adult tissue extracts to stimulate the growth of connective tissue in culture. This was confirmed by Walton (33) and by Carrel and Ebeling (5). Recently a series of papers by Hoffman and Doljanski (13, 18, 19, 20, 22) has recalled attention to the growth-activating effects of adult tissue extracts. These authors found that extracts of stable mitotically inactive tissues, such as heart or brain, were as active or more active than embryo extracts in promoting the growth of homologous fibroblast colonies in vitro. The work of Trowell and Wilmer (32) also emphasizes the activity of adult tissue extracts. Many applications have been reported of embryo extracts to wound healing (cf. Arey, 1; and Fischer, 17) and studies have recently been made of the use of adult tissue extracts in wound healing (Doljanski et al., 14; Werner and Doljanski, 34; Mandl, 27; Mandl and Maybaum, 28). It has recently been reported that neither adult nor embryo chicken tissue extracts affect the rate of healing of skin wounds of rats (Doljanski and Auerbach, 12). It might be expected that tumor tissues would contain large amounts of growth stimulating substances. Extracts of tumor tissues have been found to possess growth promoting action *in vitro* (Carrel, 3; Carrel and Burrows, 4; Bisceglie, 2; Drew, 16; Mottram, 29), but Hoffman, Tenenbaum and Doljanski (21) have pointed out that extracts of Rous sarcoma usually had weaker growth activating effect than extracts of adult muscle, and they later stated that tumor extracts affect the rate of cell growth *in vitro* in essentially the same manner as extracts of normal tissues (15).

In our work on "wound hormones" (cf. Cook and Fardon, 9, for review) we have made extensive use of yeast proliferation as a quantitative means of evaluating growth promoting activity. While this method of study has been employed more widely for "wound hormones" derived from injured yeast cells, it has also been used with success in assaying products from animal tissues (Loofbourow *et al.*, 26). It has therefore seemed of interest to evaluate the proliferation-promoting activities of embryo, adult, and tumor tissue extracts by means of their effects on yeast proliferation. Because of the quantitative nature of the yeast assays, as compared with tissue culture, the former should be of added value.

Experimental

1. *Preparation of Tissue Extracts*

Adult tissue extracts were prepared of the following organs: brain, heart, spleen, liver, kidney, and testis. The organs from 40 male Rockland mice about one month old, were each ground with an Apalant grinder and the resulting pulp was suspended in an equal volume of sterile 0.9 per cent sodium chloride and centrifuged. The supernatent liquids were filtered through Seitz filters. A mixed extract of the tissues from seven mice was also prepared in a similar manner for one set of experiments. Embryo extract was prepared in the same way from 24 embryos obtained from pregnant Rockland mice. Eight of these embryos were almost at term and the others were approximately 8-10 days old. An extract was also prepared similarly from 34 7-day old chick embryos. A tumor extract was prepared from 11 mammary adenocarcinomas (15091a) grown from tumor emulsion inoculated subcutaneously into ABC mice two to three weeks previously. Another extract was prepared from 16 similar tumors which were 16 days old. A third tumor extract was prepared from 15 11-day old dbrB mammary carcinomas grown in dba mice. The solid content of each extract was determined by evaporation of 1 ml. to dryness in an oven 85° C. The salt content of each extract was determined by titration of an aliquot with 0.1 N silver nitrate, using dichlorofluorescein as an indicator. The salt content, which was generally 10 mg. or under per ml. in the final extracts, was deducted from the dry weight in presenting the results of the yeast growth assays.

2. *Yeast Growth Assays*

These were carried out according to the rocker-tube technique previously employed in our laboratories (Cook and Cronin, 6). *Saccharomyces cerevisiae,* grown from a single cell isolated from Fleischmann's bakers' yeast, was washed from 72-hour slants on Sabouraud's medium and made up in isotonic salt solution in such a concentration that 1 ml. of the yeast suspension and 1 ml. of the tissue extract, when diluted with Williams' medium (Williams and Saunders, 35) to 25 ml. in the rocker-tube, gave a yeast concentration of 0.03 mg./ml., dry weight. The concentration of yeast used for seeding and the crop after 24 hours at 30° C. were determined by means of a photoelectric densitometer. All experiments were run in duplicate.

Results

The growth effects of the extracts are shown in Figure 1 in which the 24-hour yeast crop is plotted against the dry weight of the extract added. It is seen that the mouse embryo extract is a potent stimulator of yeast proliferation but is equalled or exceeded by the adult brain and liver extracts except in some of the lower concentrations. Heart and kidney are very active in the low concentrations but do not compare with brain, liver, and embryo in the higher concentrations, and heart falls off rapidly. Spleen and testis show relatively low activity over the whole concentration range. The extract of combined adult tissues occupies an intermediate position, as might be expected. Extracts of embryo and mixed adult tissues, when assayed in Reader's medium (Reader, 30), gave results qualitatively similar to those obtained in Williams' medium. Total growth was less, as would be expected from the simpler nature of Reader's medium.

The extract of dbrB tumors exhibited the lowest growth activity of all of the extracts studied. The extracts of tumor 15091a occupy an intermediate position and are of relatively low activity in the lower concentrations. The growth effects of only one 15091a tumor extract are shown in the graph, both extracts of this tumor having given nearly identical results.

While the chick embryo extract exhibits the greatest potency of any of the extracts, it is of interest that no special growth promoting activity is possessed by extracts of mitotically active tissues of the mouse as compared with extracts of homologous mitotically stable

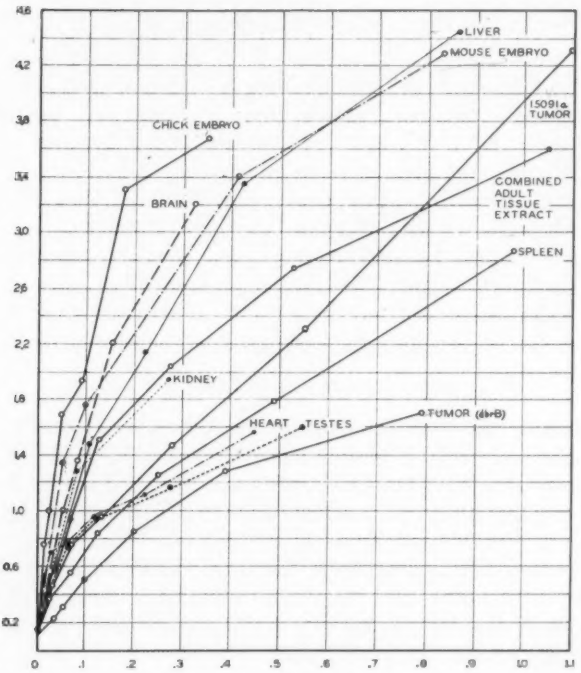

FIGURE 1

EFFECT OF TISSUE EXTRACTS ON 24-HOUR YEAST CROP IN WILLIAMS' MEDIUM
Abscissa: Tissue extract concentration, mg. per ml.
Ordinate: Yeast crop after 24 hours, mg. per ml.

tissues. This confirms the findings of Hoffman and Doljanski on tissue cultures.

Little evidence as to the nature of the active substances is provided by the present work. Ultraviolet absorption spectra of four of the extracts are shown in Figure 2. The spectra were obtained with a Beckman Model DUV spectrophotometer by Sister Miriam Michael Stimson, O.P., whom we wish to thank. Extracts of mouse tumor (15091a) and testis, as well as chick embryo, possess absorption characteristics suggestive of amino acids, while the mouse liver extract spectrum is reminiscent of nucleoprotein. The extracts in general show less selective absorption at 2600 A° than yeast extracts and much less absorption at this wavelength than "wound hormone"

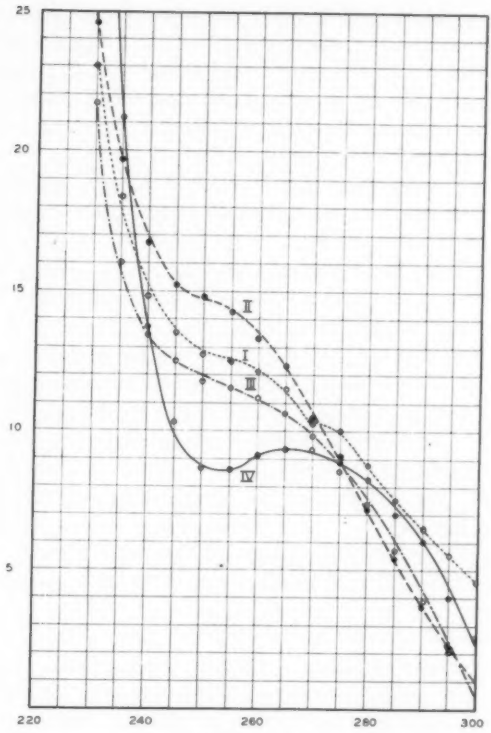

FIGURE 2

ULTRAVIOLET ABSORPTION SPECTRA OF TISSUE EXTRACTS: I. MOUSE ADENOCARCINOMA 15091A; II. CHICK EMBRYO; III. MOUSE TESTIS; IV. MOUSE LIVER

Abscissa: Wavelength in mμ.

Ordinate: $E \frac{1\%}{1\ cm.}$

preparations from injured yeast or animal tissue cells (10, 11, 25, 26). It is possible that substances in addition to the known growth factors for yeast may be operating in the tissue extracts since we also tested the brain and spleen extracts for their effects on yeast growth in Williams' medium supplemented with inositol, thiamin, biotin, pantothenic acid, pyridoxin, riboflavin, uracil, choline, acetylcholine, ethanolamine, nicotinic acid, and a casein hydrolysate (cf. Cook and Cronin, 6, 7 for the medium). These extracts showed strong growth promoting activity in the supplemented medium, suggesting

the presence of additional factors. On the other hand, Leonian and Lilly (23) have stated that the yeast growth activity commonly attributed to unknown factors in yeast and tissue extracts is non-existent and may be duplicated by adding greatly increased quantities of inositol, biotin, and pantothenic acid. In studies on yeast "wound hormones" we confirmed this statement in part (Cook, Cronin, Kreke and Walsh, 8) but reported that a residual growth accelerating activity was found in the wound hormone preparation when studied by the 24-hour yeast crop method. Evidence does show that a 72-hour growth period, used by Leonian and Lilly, may greatly diminish or obliterate the "wound hormone" effects which therefore seem to be exerted upon rate of growth rather than upon final crop. This very probably indicates synthesis of the added factors during prolonged growth periods. The importance of the incubation period in determining the growth response of yeasts has recently been reemphasized by Rogosa (31) who finds, however, no additional growth effects to be caused by yeast or tissue extracts under his conditions and who concludes that "the 'bios' question is probably solved." Since Loofbourow (24) and we (8) have demonstrated effects of adenine and guanine nucleotides and nucleosides on the early growth of yeast, we are inclined to consider that the question is at least partly open.

Summary

Extracts of mouse embryo, of adult mouse brain, heart, spleen, liver, kidney, and testis, of mouse adenocarcinomas 15091a and dbrB, and of 7-day chick embryos were tested for proliferation-promoting effects on yeast (*S. cerevisiae*) in Williams' medium. All extracts contained growth-active substances but brain, liver, and embryo were most potent on an equal weight basis. Extracts of mitotically active tissues did not possess unusual proliferation-promoting activity. Extracts of chick embryos caused greater growth than either adult or embryo mouse tissue extracts.

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Growth, 1944, **8**, 259-309.

THE ORGANIZATION OF PROTOPLASM

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(Received for publication on September 20, 1944)

A. INTRODUCTION

From the wheel and the lever man has progressed with his inventions to electric generators and robots that give him remote control over useful forces in his environment. His machines, which tend toward automatism, have suggested to him that organization exists in different degrees in the visible creation, the highest degree being in organisms.

The perfection of a mechanical device gives evidence of the intelligence of the inventor. The degree of intelligence attained by an ancient people is recognized in the remains of their creations. Just as the archeologist discerns the culture of a people by their works, so may we discover the image of God in the visible creation. In the phyla, an increasing complexity is recognized in the form and function of organism and also in the various phases through which an organism passes during its ontogenetic development. There also is greater specialization of form and function. The complexity increases. Though the organization is more evident to the eye in the higher organisms, the organization of the protoplasm is just as complex in unicellular as it is in multicellular organisms. The organization in any organism must ultimately be sought in the protoplasm. In the protoplasm, in the tissues, organs, and in the organism as a whole, a remarkable correlation of the parts and their functions is evident. The organization is indicative of design. We discern the Hand of the Master Builder in organization.

As man acquires more knowledge and the discovered facts are integrated, the notion of purpose in the functions of cells, tissues, and organs cannot escape him. One must do violence to his reason to deny it. It is not logical to ignore that which is not strictly demonstrable with the aid of the limited facts that we have thus far established in the domains of matter and energy. It follows that

teleology cannot reasonably be ignored since we can often discover the function from the mechanical arrangement and operation. Sometimes the mechanical explanation is not clear but the teleological explanation is evident and vice versa.

As our mental horizon expands, the contact with the mysterious unknown must increase and produce a sense of wonder and awe. This culminates in an act of adoration of Almighty God. Such an act is not superstition, or the result of mental weakness, but is a just tribute to the Almighty.

There is today a demand for simplification. Efforts constantly are being made to present scientific facts and theories in familiar terms. There is a limit to simplification and some mental effort on the part of the reader must be demanded to make the proposition clear to him. Often enough the simplification degrades the proposition to the point where evident overstatements and understatements are made. Overstatements and understatements are heretical in science as well as in religion. An effort was made to present the matter here in the simplest possible terms in order that those who are not scientists may be able to read this thesis with profit. There is no purpose in stating interesting facts and drawing images to arouse the imagination merely to amuse. An effort is made here to integrate the facts regarding the structure, physics, chemistry, biochemistry, and physiology of protoplasm and thus demonstrate its organization. Special attention is given to those mysterious biochemical regulators, enzymes, hormones, vitamins, phytohormones, and evocators which form an intrinsic part of the organization and are indispensable in the functioning of organisms.

The ontogenetic development of the organism gives unequivocal evidence of the organization of the protoplasm since the phases do not follow each other in a haphazard manner but in a directed manner with definite purpose. It is pointed out that this organization is not to be sought in any one cell, tissue, or organ, but in the protoplasm itself. From the moment of fertilization, to maturity, the organization is in the protoplasm acting in the organism as a whole. Our limited and biased notions of location in time and space make it difficult to comprehend fully this fact.

There is a definite trend away from the materialistic viewpoints of classical physics and chemistry which dominated the schools during

the past century. No similar trend has been noted in biology where admittedly we are dealing with systems far more complex than are inorganic systems.

The most striking evidence of organization in the protoplasm is given by the adaptive functions, by means of which adverse factors in the environment can be made innocuous when they threaten the existence of the individual organism or the survival of the species. When the existence of the organism is threatened, as in disease, certain adaptive measures, called "symptoms," come into action. These symptoms may be regarded as the sum of these adaptations and may involve profound changes in the organization.

The organization may become impaired and the powers of adaptation may be reduced. The power of resisting disease may be lowered accordingly. It is suggested that our urbanized life causes man to be less robust and that some of the modern ills that are claiming victims at an increased rate have their origin in the disturbance of the dynamic equilibrium that should prevail.

B. Organization in General

The term "organization" is not easily defined but we obtain a satisfactory notion of its meaning if we consider the sum of human experience with machines and groups of people who are associated in some kind of society. It is not difficult to distinguish between a machine or society which is well-organized and one that is disorganized. In the well-organized machine or society the end in view is achieved readily because the individual parts are fitted to fulfill some definite function required of them and the activities of the parts are coordinated in such a manner that the functions of the parts are complementary and the end in view is attained efficiently. In a disorganized machine or society, the fault may be due to a lack of efficiency of one or more of the parts or to a lack of coördination of functions.

Thus, independently operating parts may be so arranged that their functions become interdependent and complementary. The end in view is achieved not by the operation of one part independently but by the combination of all of the functions which are directed toward a common end. A watch, the purpose of which is to indicate the time, must have its parts properly designed and arranged and made

interdependent and complementary before it can fulfill its function. Each part has its own function which, however, has no purpose unless it is the complement of the other functions which have as an end the indication of time. So also raw recruits can be drilled and trained so that their coördinated and combined activities may win a victory from the enemy. Again, a group of men may become associated in an efficient business organization.

With the progress of civilization the notion of organization in society and its importance in attaining the general good of the individuals and society as a whole, is constantly more appreciated. The governing bodies learned in the past to arrange and connect the arbitrary activities of the individual human beings into regulated and dependent activities which now work together for the general good. Society has no reason to exist unless it achieves the good of the individuals who are associated. From this it is clear that the functions of the individual are at once means and end.

The problems of the relatively young science of sociology are primarily problems of organization. The large number of barbarians and semi-barbarians and the general unrest in civilized groups of men today are sufficient evidence that the final and best formula for the most efficient organization in human society has not been discovered. In general it might be said at this time that the position of the proletarian is nearly as low as was that of the serf in feudalism. He despairs of escaping from bondage and of having sufficient power for self expression. For a time at least in the past the artisan was, or could be, the head of his own shop; the farmer could own his own field; the fisherman his own boat. Before end and means became antagonistic as we now find them in the functions of the men associated, men worked hard for the common good, took pride in their achievement, having an incentive to coöperate with their fellows to achieve the common good. Today, the laborer and the clerk might enjoy similar liberty of self expression and maintain their dignity in their profession, if there were better organization in society and it were not necessary to measure all activities in functions of money. If mass production cannot be carried out without sacrificing the development of the individual, then this method of serving society is not satisfactory because it fails to recognize that in a satisfactory organization the individual must be end as well as means. When this

fact is not recognized in an industrial civilization, the faulty organization and not the civilized indivdual must be sacrificed. Our civilization, with its many failures and few successes, has taught us the great need for organization.

The first scientist to detect organization in organisms was Lavosier. He lived in a period when society was in the process of reorganization and was organization-conscious. No wonder that he detected organization in the make-up of plants and animals. He compared organisms with machines. Machines to his mind best expressed the notion of the organization which was so evident to him in living matter. Since his time many comparisons have been made between organisms and various kinds of organized devices, notably with groups of human beings. Thus, for example, an organism that does not manifest a high degree of differentiation and specialization in its cells and tissues and organs has been compared to the small town where civic organization is primitive but sufficient to take care of its needs. The individual members of such a group of men do not have highly specialized functions and can serve in many different capacities simultaneously. In the modern large city the organization must necessarily be far more complex. The individual members of the organization must be restricted in the kind of work they do, that is, their functions become specialized. The organization of highly differentiated plants and animals resembles that of a large modern city because its cells and tissues are greatly differentiated and specialized but well grouped and associated with each other so as to make their functions complementary. A complex modern city is therefore used to illustrate the well-organized plant or animal in which the parts are highly specialized in form and function to form a smoothly operating complex in which a definite end is achieved.

The organization in the plant and animal, so definite and absolute, was overlooked for a long time. Much attention was given to the description of the general form, the systematic arrangement of the organisms in phyla, detailed accounts of their anatomy and histology, and too little attention was paid to the correlated activities of the constituent parts or the organization that is the most characteristic mark of organisms.

Organization may be defined as the arrangement of interdependent parts of a complex in such a manner that the function of each part is

in proper relation with the functions of the whole, so that the whole complex is systematized into such working order that a projected end may be achieved.

In the study of plants and animals, anatomy and histology suggest organization, but in physiology the functions of cells, tissues, and organs are studied and it becomes evident that the orderly arrangement of the parts, as reported in anatomy and histology, is meaningless unless the functions, the ends to which the arranged parts are directed, are recognized. Physiology itself is meaningless unless design, purposive arrangement, and function in the organism are admitted.

Organization is the most significant mark by which living things are distinguished from inanimate matter. Plants and animals are called organisms precisely because they are organized.

The doctrine of organicism maintains that the processes, which are characteristic of living things, are the manifestations of activity possible only in virtue of the state of autonomous organization of the system rather than because of the individual components. Some who subscribe to this doctrine hold that organization merely is the result of an inherent property of matter which can adapt itself to circumstances and exclude any notion of design. This view is a pure assumption and there is no satisfactory evidence to support it. In biology there are many evidences of design in the behavior of organisms and the characteristic processes of their lives. The organs of the living animal body, for example, are correlated by the organic fluids and the nervous system. Each element of the body adjusts itself to the others and the others to it. This kind of adaptation is not noted anywhere in non-living things. In the inanimate world adjustment is limited as expressed in the law of mobile equilibrium. According to this law, the alteration of one of the factors determining the state of equilibrium in a system, which is capable of internal adjustment, is accompanied by a change which tends to annul the effect of the original alteration.

A kind of organization, which is of a lower order than that which is referred to in speaking of animals and plants, exists in the inanimate world. This organization is of different degrees and care must be taken in speaking of it to distinguish what level is involved. Electronic, and molecular structural levels can be distinguished and also

higher levels dealing with concepts of mass, force, energy, and entropy. Still higher levels are reached when dealing with molecular aggregates of special structure. In dealing with the organization of plants and animals, highest level is reached. There the organization concepts of the cell, the tissue, the organ, the organism as a whole, the biological systems, the chromosomes, the genes, the heredity, the reflexes, etc., are involved and cannot be explained as long as only the lower levels are considered. Since the organism has a physical basis of life, it is evident that all of the organization recognized at the lower levels also exists in the organism, but it is the degree of organization which is higher than all of these and cannot be reduced to these that is characteristic of the organism. The recognition of this fact is presupposed in the science of physiology. The notion of function there expressed has no meaning unless it is admitted that organization on a level, above that noted in physics, chemistry, and physical chemistry, is recognized.

The highest degree of organization is clearly manifested in the process of adaptation which cannot be explained by the law of mobile equilibrium. Adaptation is a process of adjustment by organisms to their environment so as to favor the survival of the organism or to make an adverse factor innocuous. This process is characteristic of physiological processes. It assumes as many forms as there are functions in cells, tissues, organs, and organisms. These many forms may be arranged in two categories; intraorganic adaptations and extraorganic adaptations.

The constancy of the organic medium and the relations of tissues and humors are brought about by intraorganic adaptations. The correlations of organs are determined by this kind of adaptation. It is also responsible for the repair of injured tissues and the cure of disease. Extraorganic adaptations adjust the organism as a whole to the physical environment in which it is immersed. Extraorganic adaptations make it possible that the organism can survive in spite of adverse factors by making them innocuous.

Intraorganic adaptation is essentially teleological. The physiological processes associate themselves in a purposeful manner to attain a definite end. Each part works toward a common end to satisfy some need which may exist or may arise at some future time. This is demonstrated clearly by the changes that take place in the

pregnant mammalian body just before parturition. The tissues and organs are prepared before this to make the birth and sustenance of the offspring possible. In the plant world the same principle of anticipation is illustrated by the casting off of the leaves by deciduous plants before winter begins. At the time of the first frost the anatomical arrangement by which this is accomplished is completed and the trees and shrubs become bare. While they act like hygrophytes in spring, summer, and fall, they act like xerophytes in winter. If this arrangement were not made, the plants would dry out during the winter and die.

The metabolism of organisms, their degree of locomotion in relation to their awareness of environmental factors, their consequent response to these factors, growth, regeneration, reproduction, and euphory, all point to an organization that is far beyond anything encountered on lower levels and cannot be reduced to the organization of those levels.

C. The Physico-Chemical Organization of the Protoplasm

In multicellular and unicellular organisms the protoplasm is the essential part of the cell. It is divided into cytoplasm and nucleoplasm. The physiological activities of the cytoplasm are regulated by the diminutive nucleoplasm. Except during the period of cell division, the two remain separated. However, a constant exchange of material goes on between them keeping the whole in a state of dynamic equilibrium. The apparently independent masses of protoplasm of the cells forming tissues in the multicellular organisms are coöperative units in an organized whole. They are immersed in lymph in the animal body which serves as a medium of exchange. In the plant body the photoplasmic masses are, in some cases at least, connected by strands of protoplasm called plasmodesms. The constituent parts of the protoplasm itself operate in interdependent manner to make the functioning of the cell as a whole possible. The protoplasm of the unicellular organism is not less organized than is that of the multicellular organism even though it is not subdivided by septa. It is less specialized than it is in the cell of a tissue, which, being specialized and having only restricted functions, is really simpler than the generalized protoplasm of the protozoa and protophyta.

The protoplasm is composed of 14 of the 92 known elements with traces of four more. It is the fact that these elements, which occur

in both the inorganic as well as the organic world, form a biological system of related parts that is significant. The more complex bio-substances usually are found in systems of molecules which are referred to as giant bio-molecules. The constituents of protoplasm are water, proteins, fats, other organic substances, and inorganic material. Eighty-five per cent is about the amount of water usually present. Ten per cent is about the amount of protein, two per cent the amount of fatty substances, one and a half per cent the amount of other organic substances, and one and a half per cent the amount of inorganic material present. The relative number of molecules determined from the average molecular weight of the substances would be as follows: Protein = one; water = eighteen thousand; fatty substances = ten; other organic substances = twenty; inorganic material = one hundred. This gives a pretty good picture of the enormous size of the protein molecule as it occurs in the protoplasm. Water is the smallest molecule but has characteristics peculiar to itself. Each molecule consists of one atom of oxygen and two of hydrogen. The atomic nuclei and constituent electrons are so arranged that two residual negative electrical fields and two positive fields occur at tetrahedral points in the molecule. The result of this arrangement is that the water molecule is a permanent dipole. The consequence is that when a portion of the water molecule occurs as a hydroxyl group on the organic molecule, one of the positive residual points is missing due to the formation of the primary valence bond. This leaves three points for coördination with the water molecules in the surrounding medium. The negative residual charges on the water molecule are due to the nature of the oxygen. When an (OH) group occurs on an organic molecule one of the positive residual points is missing due to the formation of the primary valence bond; this leaves only three points for coördination with water molecules in the aqueous medium. The only other atom in the protoplasm which is similar to the oxygen in this respect is the nitrogen atom. It exhibits only one negative residual charge. It follows that the same may be said of the (NH) and the (NH_2) groups as has been said for the (OH) group. Residual charges of this kind are lacking in the carbon atom and in the (CH_2) group. The closer approach of the atomic nuclei in the water molecule and in the protein molecule, due to the attraction of the hydrogen nucleus by the residual

negative on the oxygen or nitrogen, is called the hydrogen bridge. The hydrogen bridge then is caused by the residual charges on oxygen and nitrogen and the lack of such on carbon. The relationship between the water and the protein in the protoplasm is largely due to the hydrogen bridge.

All of the proteins are built around the same structural scheme. The amino acids are of the alpha form. They link to form a long peptide chain in which the adjacent residues alternate from side to side. The chain forms a zig-zag to form a "back-bone." Amino acid residues may form various side chains producing a kind of arrangement which is responsible for various specific qualities of the protein. The dimensions of the basic structural chains are about the same for all. Each amino acid residue is about 3.5 Å or less in diameter. The length of the chain depends on the total number of residues present. If for example, 300 residual chains are present the chain would be approximately 1,000 Å long. The shortest residual chain, glycine, is hardly 1 Å long and the longest, arginine, 8 Å long. The width depends on the average. It is fairly consistent and is about 10 Å. The thickness through the "back-bone" perpendicular to the plane in which the width is 10 Å is about 4.5 Å. Thus the molecule having 300 residues may be pictured as 1,000 Å long, 10 Å wide, and 4.5 Å thick with a molecular weight of 36,000. Some of the protein molecules are globular in outline rather than elongated. The term globular would here include shapes ranging from the sphere up to considerable elongation. The longer chains seem to be folded into packets so that a chain of the dimensions given, would make a block having 20 folds each about 50 Å in length arranged into three layers of about seven in a row. The length and the breadth of the block would be about 30 Å.

The hydrogen bridge would make such packets take on water molecules wherever oxygen- and nitrogen-containing groups presented themselves to the aqueous medium. Hydrated particles probably are bound to similarly hydrated particles by means of the hydrogen bridge at the adjacent "back-bones."

Ionic forces may be effective between oppositely charged groups. Such forces that combine particles might also be the resultant of several or many charges. In this way particles may be held together by positively and negatively charged atoms and by the resultant field produced by all of the charges on the particles whether they lie on the surface or not.

For every protein giant molecule there are at least 10 or 15 molecules of fatty materials which play a rôle in the general organization even though they do not contribute much to the physical strength of the structure. These units are characterized by the excessive number of CH groups and the relatively few oxygen and nitrogen atoms. In this group are long chains of fatty acids, neutral fats which are glycerol esters of the long chain fatty acids, phospholipids and sterols. The phospholipids and sterols are present in almost constant quantities which indicates a well-balanced state. They are associated closely with the proteins of the heavier proteins of the heavier granules. The best known phospholipids are the lecithins and the cephalins. About half of the phospholipids in the protoplasm are lecithin and the other half are cephalin.

The proteins form complexes with sugars and acids and so become molecules of considerable size. The nucleoproteins form such large complex molecules. They are present in the nucleus and in the cytoplasm as well. Nucleoproteins are particularly abundant where cell proliferation is in progress as for example in actively growing tissues and in the meristems of plants. The chromatin substance is almost entirely composed of nucleoproteins. There are many problems that seem to have no solution until the chemistry of the nucleoproteins is better known.

Paired particles in the chromosomes have been recognized as the carriers of hereditary characters and have been called "genes." Classical genetics concerned itself with the mechanism by which the transmission is made possible. The biochemical approach is to attempt to show how the genes produce their characteristic effects. The problem is attacked from the lower level of biochemical reactions and not from the higher level of structural and functional organization. The chemical analyses of the chromatin of sperms suggest that the genes consist mainly of thymonucleic acid combined with histones and protamines. Staining and ultra violet absorption methods lead to the con-

clusion that the nucleic acid is concentrated in dark-staining bands. Enzyme digestion experiments show that nuclease breaks down the nucleic acid components but does not destroy the continuity of the chromosomes. Trypsin does destroy the basic structure so that it is probable that the continuity depends on histones and protamines. The sum of biochemical research makes it probable that the genes are specific protein molecules or groups of molecules possessing the power of reproduction under appropriate conditions in the living cell. It is supposed that they have as primary function to control protein specificities, possibly by serving as models. It is possible that each enzymatically catalyzed reaction is under the control of one particular gene. It is generally agreed that the gene must be regarded as an integral part of the protoplasmic biochemical system.

Water and the proteins are the most important constituents from a structural consideration. In order of relative importance follow the fats—including sterols and lipids—then come the inorganic substances. Least in importance are the carbohydrates. They represent mainly stores of fuel to furnish the necessary energy in the organization.

The animal cell usually is self-limited without a visible membrane. On the contrary, most of the plant cells are of definite shape and are bounded by a cellulose membrane. This membrane, though dead, is important in the general organization of the plant.

In some primitive organisms the protoplasm does not develop septa that subdivide the total mass of the protoplasm into cells, but the nucleoplasm breaks into many nuclei. Such an organism is called a syncytium or coenocyte. It is a matter of choice if it is to be regarded a multicellular organism lacking septa or a unicellular organism with many nuclei.

When the protoplasm of multicellular protoplasm forms septa and the cells multiply and differentiate, the daughter cells taking on different forms and functions, the directing influence of the process must be sought in the protoplasm. The influence which makes like cells assemble to form different tissues with specific functions must reside in the protoplasm. Even though the protoplasm of the individual specialized cells in a tissue appear to have lost many of their powers, tissue culture has revealed that many of the supposed powers are really suspended, merely dormant, and the potential powers be-

come actual under unusual environmental conditions. When, for example, the organic medium undergoes physico-chemical change as the result of disease, the tissue cells manifest new powers. It is the functional characteristics more than the structural that reveal the high degree of organization in the protoplasm. It is the organization that makes it possible for the physical basis of life to build and sustain the body of the organism. True fats serve mainly as fuel to supply energy in the organization. The sterols and compound lipids do not supply much energy but have functional value in the protoplasm.

The physiological approach to the investigation of living things and their organization has taught us that the phenomena of life cannot be understood from the study of carcasses alone. It has also shown that cells must be studied in proper media so that they will exhibit the functions which give evidence of their organization. The physico-chemical approach does, however, help to discover the organization by showing the vehicles by means of which the organization is made possible.

Thanks to modern methods, living cells can be seen in operation, while immersed in satisfactory media. All living cells modify their proper media and unless excessive modification is prevented, a given medium soon becomes unfit and the cells die. The structure and the function of the protoplasm are therefore not independent of their environment but dependent upon the physico-chemical factors in the medium. From this fact it is clear that the study of anatomy, histology, and cytology are not sufficient to give us complete and satisfactory knowledge of the living cell and its organization.

The living cell operating in a proper medium, may be pictured as an active granular semi-viscous hyaline mass within which moves or drifts an ovoid, elastic-walled, balloon-like body called the nucleus. This nucleus appears to be filled with a transparent jelly. Within the nucleus there are two very small, constantly-moving ever-changing nucleoli. Within the cytoplasm and around the nucleus many of the granules can be seen to be in great agitation. In another part of the cytoplasm in which is located the apparatus called the Golgi body, the granules are most unusually active. Many of the finer granules revolve in small circles, never going beyond a certain range, but remaining in a definite field in the cytoplasm. Larger globules endlessly zigzag through the cytoplasm without fixed limits. Filaments—some-

times long like snakes, sometimes short like bacteria—whose functions are not known, move freely in the cytoplasm. The general mass of protoplasm moves around in the cell in a more or less regular orbit; this is called cyclosis. The mechanics of this motion is not known. The cytoplasm also has different streams resembling the ocean currents which course through the cytoplasm in different directions. The streams of hyaloplasm have their directions indicated by the particles that are immersed in it.

The fixation of the protoplasm as carried out in the staining methods of histology destroys its structure and produces artifacts. Much controversy arose in the past regarding the interpretation of these artifacts. They do indicate the presence of certain structural arrangements but they cannot give convincing evidence of the actual relationships of the parts involved in the operative whole, which is the necessary requirement that the definite function be fulfilled. The fibrillar and reticular structures obtained in the coagulum are probably visible fibrous entanglements of submicroscopic linear molecules. It is not likely that the forms which do manifest a degree of regularity should be the result of finely, arbitrarily distributed globules and granules in suspension. These final forms are interpreted as evidence of submicroscopic threads which are present in the living protoplasm.

The structure of the protoplasm is not as simple as it was at first imagined to be. A careful study of the physics of the protoplasm is constantly revealing new facts which are helpful in improving our knowledge of its organization.

Water is the dispersion medium and salts dissolved in it modify the physical qualities of the protoplasm. Sugars dissolved in the water release the energy used in the aerobic and anaerobic metabolic processes. Fats serve as sources of reserve energy. Protein furnishes the wherewithal to build the structural framework of the physical basis of life. Vitamins, enzymes, and hormones are chemical regulators of the physiological processes. The physics and the chemistry of the protoplasm are of great importance, but of even greater importance is an understanding of the arrangement of the parts to make the functioning of the whole possible. Much more needs to be learned about the organization of the protoplasm which makes it a dynamic biological system.

For a better understanding, the metabolic processes, the composition,

the structure, the energy relations, and the organization must all be better known.

One of the primary attributes of protoplasm is its power of utilizing energy in the process of building substance of its own kind. This is the fundamental notion of growth. The notable thing about this process is that matter with an organization of a lower order is transformed into living matter which has an organization of a higher order. The essential synthesis involved in this process utilizes energy, but in the synthesis found in the organization of the lower order energy is released.

Oxidation of carbohydrates furnishes most of the energy utilized by the protoplasm. This oxidation is in itself not as significant as the uniqueness with which the released energy is utilized. It is this that distinguishes the energy relations of living systems from the relations encountered in non-living systems. This characteristic property depends no doubt upon the arrangement of the parts of the protoplasm as well as upon the chemical relationships; in a word, upon the entire organization. Chemical analysis of the protoplasm fell short of what was at first expected of it because the organization was not taken into consideration.

The stereochemist was not at first taken seriously because links and chains, rings and side chains, amino groups and carboxyl groups which he attached here and there were not made visible. Confidence in his theory was merited because it gave lucid explanations of hitherto obscure relationships and of evident, previously unexplained facts. In the same manner the biologist who accepts the theory of sterometry has had his confidence justified that the structure of the protoplasm and its organization will be understood better if he interprets his results in the light of this theory.

Every honored theory, even though eventually discarded, contributes something to the body of our knowledge. There always is something of the truth in it which persists. The emulsion theory of the structure of protoplasm overstated the facts when it claimed that nothing but a grouping of unit spherical units of structure properly dispersed in a liquid phase was required to explain the structure of protoplasm. The presence of globules, alveoli, and micellae was explained in this manner. It was attempted to explain the general behavior of the protoplasm on the same basis. It is true that pressure-

symmetrically-arranged globules in the protoplasm assume the shape of dodecahedra or tetrakaidecahedra with hexagonal cross-section, and the evidence is sufficient to prove that spherical shapes are present in protoplasm in the suspended state and that all of the particles present are suspended in such a manner that the protoplasm resembles emulsions in many ways. It is not true that this is the sum of all of the facts about the structure of protoplasm. It is well known now that protoplasm is something more than an ordinary emulsion in which spherical bodies are suspended in a liquid phase. The qualities of a substance, that is nothing more than an ordinary emulsion, satisfy very few of the requirements to form the living system called protoplasm. The protoplasm has physical qualities which ordinary emulsions do not manifest. Ordinary emulsions are not elastic and they do not imbibe water as protoplasm does. What emulsions and protoplasm have in common is a stabilizer which prevents both from breaking down. In the protoplasm this is a protein stabilizer. In milk the stabilizer is also a protein. When the protein stabilizer coagulates in milk the suspended fat globules separate out from the aqueous phase. In the same way the structure of the protoplasm breaks down when the protein stabilizer coagulates. It is the stabilizer which makes the protoplasm resemble an emulsion. Clues to the submicroscopic structure of protoplasm are given by some of its physical, chemical, and biological qualities. The investigation of the elasticity, tensile strength, contractility, thixotropic behavior, gelation, fluidity, streaming, cyclosis, symmetry and asymmetry, spirality, crystallinity and selectivity has led to some important conclusions regarding the structure of protoplasm.

The data obtained in recent investigations have enriched the body of evidence compiled by the early students of protoplasm which proves that the physical basis of life is truly elastic. The simplest convincing proof is given by the recoil of the protoplasm in assuming its original shape when released from the microneedles that distorted it.

The degree of elasticity is not a fixed value in protoplasm. For this reason no satisfactory elastic constants can be given for it. The values that have been expressed for it at given times are surprisingly high. It is not surprising that the degree of elasticity is variable, because the protoplasm is a thing that is labile, being constantly in flux.

The viscosity of the protoplasm also is variable. Apparently the elasticity and the viscosity have no direct bearing on each other. Highly viscous protoplasm is sometimes poorly elastic, and rather plastic. Sometimes a thin protoplasm is surprisingly extensible and has a considerable recoil. Salts affect the elasticity of the protoplasm, for example, calcium increases its extensibility whereas sodium reduces it.

The structural features of the protoplasm, as in gels, must be made responsible for its elastic qualities. In gels the elasticity was ascribed to surface tension but the assumption is questioned now because the elasticity is not restricted to the surface. If the gels manifest a greater degree of elasticity on the surface, the cause should be sought in the fact that the fibrous molecular aggregates are denser and more closely knitted on the surface.

In colloid chemistry an attempt was made to explain the elasticity of protoplasm with the brush-heap theory. This theory assumes that rods of molecular aggregates are distributed in a haphazard fashion in such a manner that the whole mass holds together. Such a distribution is possible and might help to explain the elasticity up to a certain point but theory fails when it is attempted to explain some of the other physical qualities of protoplasm by it. The best explanation thus far given for the manifestation of the elastic qualities is the assumption that the protein molecules are folded. Good evidence has been adduced to show that folded molecules exist in albumin, wool, hair, and muscle tissue.

A folded protein chain may well be the basic mechanism by means of which the protoplasm becomes elastic and manifests contractility. It is, however, possible that the modified helix thought to exist in rubber, also is present in the protoplasm. It was for a time thought to be impossible that a hydrocarbon should be elastic but now it is known to be a fact that elastic artificial rubber is made up of polymerized hydrocarbons.

The extended chain is limited to one geometrical form. The contracted chain can occur in many varied forms, and it has a tendency to contract. This appears to be true for all synthetic polymers. It does not seem to be too daring to assume that it is true for protoplasm in general and in muscle tissue in particular. Most chemists agree that polypeptid chains, with side chain linkages form two and

three dimensional lattices in the proteins. It thus seems probable that there are such forms present in the protoplasm and their presence is sufficient to explain its elastic qualities. It is difficult to explain the elasticity, the tensile strength, and the imbibition of protoplasm if it is assumed that all of the molecular structures in it are spherical. Evidence is predominantly in favor of the notion that there exist in it proteins in the form of convolutions of peptid chains. It is not precluded, however, that globular proteins are simultaneously present in the soluble form as in egg albumin, insulin, and edestin. Protoplasm might well contain both globular proteins serving in the capacity of nutrients and biochemical regulators and also linear proteins serving in the capacity of units of structure.

Rhythmic contractility in living matter is of such widespread occurrence that it is probably a quality of living protoplasm. It is observed in heart muscles, the muscles of the diaphragm, the walls of the intestine, uterus, fallopian tubes, in all resting muscle cells, in the plasmodia of slime molds, and in many one-celled organisms. It is also observed in the individual cells cultured from chick-embryo heart. Probably the pulsation of the heart is the resultant of the many component pulsations of the heart-muscle cells. The different cells may be in different phases of pulsation among themselves at a given time.

The most reasonable explanation of the mechanics of the contraction of the protoplasm is that the total contraction is due to the sum of the contractions of the principal structural proteins. This probably is accomplished by the folding of molecular fibers symmetrically aligned and joined one to the other by side chains, so as to form a three-dimensional lattice. The energy required for the shortening of the protein fibers is furnished by the oxidative processes of the cell. This energy may or may not be supplied rhythmically. A cycle of oxidation-reduction reactions is assumed to pass along a nerve in the path of conduction. A number of autocyclic rhythmic reactions are known to occur in the protoplasm of cells but the conclusion is not warranted that the rhythm of protoplasmic flow and contraction is to be sought in the manner in which the energy is supplied to the vital mechanism. Whatever the explanation may be, the conclusion drawn from the evidence cannot be escaped that the rhythmic contraction of the protoplasm demands a highly organized mechanism.

Cyclosis is another natural phenomenon observed in protoplasm which is indicative of organization. Change in surface tension was long assumed to be the cause of this movement, but no one ever had great confidence in this explanation because it was never really satisfying since there are too many reasonable objections to it presented which remain unanswered.

A peculiar shuttle type of flow, first in one and then in the reverse direction can be observed in the coenocyte of slime molds. This was explained as the result of hydration at one pole and dehydration at the other. It is improbable that this is the real explanation because it continues when the protoplasm is completely immersed in water. Cataphoretic migration of particles was also advanced as a possible explanation of both this behavior and cyclosis. It was thought that the aqueous medium might then flow as the result of electroendosmosis. The difficulty in this case is the lack of any change in the electro-potential of the protoplasm or its parts while these movements occur. In the slime molds where the direction was reversed in this shuttle movement every 45 seconds no reversal of potential was noted.

Since the rhythmic contractions of the protoplasm in the plasmodia of slime molds is synchronized with the streaming, it seems likely that the two phenomena have a common cause. This cause probably is to be sought in the rhythmic contractions in the elementary structural parts of the organized mechanism.

When a gel is mechanically agitated it sometimes collapses. This phenomenon has been termed thixotropy. If it is assumed that the structure of a gel is as was described above, the explanation seems to follow naturally that the structural solid particles become dissociated and can no longer remain suspended. The same phenomenon is observed in protoplasm and the same explanation namely that the specific structural features which make the state possible are destroyed. When photoplasm is made to coagulate by similar methods, the cause is again to be sought in the destruction of the physical organization. A simple example of the phenomenon is the liquefaction produced in protoplasm when teased with needles. The agitation just as frequently causes coagulation. One-celled organisms and the eggs of some algae often disintegrate instantaneously when they are punctured. When the eggs of some Echinoderms in the stage of diffusion are subjected to pressure, they liquefy. A pathological condition

sometimes causes a similar collapse of the protoplasm. Some anesthetics produce thixotropy in the protoplasm of slime molds. When such experiments were carried out, it was noted that the protoplasmic movements ceased suddenly and gelatinization occurred simultaneously, indicating that pronounced physical and physiological changes occurred as the result of the treatment. The effect of anesthetics in the higher animals possibly is the result of thixotropic setting of the protoplasm of the nerve cells.

From the fact that thixotropic gels possess large quantities of water, it is deduced that the solid matter must have long flexible fibers as structural units. Such fibers can make contact with each other at greater distances than can spheres and consequently fewer elements are needed to make a given quantity gel. A cadmium gel, for example, contains 99.8 per cent water. By way of comparison it is interesting to note that only 2 to 3 per cent of the body of the jellyfish is solid matter. The sudden locking of the fibers might explain the gelling and the destruction of the locked state might explain the liquefaction. The thixotropic behavior of the protoplasm might be explained in the same way. Polypeptid chains are known to exist in the protoplasm and it is reasonable to think that these can act in a manner comparable to the behavior of the fibers in a thixotropic gel. This behavior of the fibers then is again an indication of a definitely organized pattern in the living protoplasm.

D. The Biochemical Organization of Protoplasm

The organization of plants and animals is manifested by all of the activities of the organism. In higher plants and animals the organization is more evident because of the high degree of differentiation of the cells, tissues, and organs. In the end, however, the organization must be referred to the protoplasm. The physical organization of the protoplasm was not generally admitted at first, but modern science and invention has made it possible to verify the fact that the physical organization extends into the minutest structural details. The protoplasm is equally well-organized chemically, but this is not as readily visualized as is the physical organization. However, the external evidences make it not less certain.

Respiration furnishes a good illustration of the manner in which external evidence proves internal organization. Oxygen is absorbed

and carbon dioxide is eliminated while the released energy enables the protoplasm to do useful work so that the vital processes can be carried on in a purposeful manner. The illustration also shows that energy must be considered in the study of the organization. It also seems evident from the data on respiration that not only are the materials that enter into the reactions and the products that result involved, but also certain biochemical factors that act as regulators are involved. The life-activities clearly point to a biochemical organization that certainly is very complex.

The protoplasm must be conceived as a heterogeneous system of substances in which the colloidal state is predominant. The dispersed organic and inorganic substances are very finely divided. The smallest divisions do not come within the dimensions which can be made visible to the human eye even with the aid of our best devices. The dispersion medium is water and aqueous solutions of organic and inorganic solutes. A protein stabilizer makes the dispersion of the particles possible and prevents the collapse of the emulsion-like state of the mixture. The water of the protoplasm does not act like water in the free state. It does not have the physical qualities of ordinary water and hence is said to be "bound." A number of facts regarding the life and adaptations of organisms point to the fact that this binding of the water is very important in the matter of the organization of the protoplasm. The state in which we find the substances mixed in the protoplasm particularly is well-adapted to the successful execution of the complicated physical, chemical, physico-chemical, and biological activities manifested by organisms. If the mixture breaks down or coagulates, the life activity ceases; diminishes in rate or is suspended. It is evident from this that the normal state of the protoplasm is a prerequisite for the proper functioning of an organism and cannot be disturbed beyond rather narrow fixed limits without endangering or even destroying life. It also follows that the protoplasm must be considered physically as a carefully and delicately balanced system of parts which are biologically related. The semiliquid mass is prevented from unlimited spreading by an elastic membrane. This membrane makes selective absorption and elimination of materials possible. This activity is called directed selective permeability. In virtue of it certain substances will permeate the interior of the cell, while others though similar in character and size fail to

do so. Likewise certain substances escape from the protoplasm while similar ones are retained. This property of the cell is well illustrated by the behavior of the cornea of the eye. The corneal cells allow the oxygen of the air to diffuse into their interior and eventually to reach the lens. But they do not permit the entrance of carbon dioxide from the air. On the other hand the oxygen of the interior of the eye does not diffuse outwardly. The carbon dioxide resulting from the metabolic processes within the lens eventually reaches the air after passage through the cornea. If the cell becomes diseased, its directed selective permeability is destroyed or is at least diminished. Senile cells are less permeable than young healthy cells and so suffer from malnutrition, failing to liberate the toxic waste product as well as they should. Selective permeability plays a major rôle in the physical processes of the organism and is clear evidence of the organization of the physical basis of life.

Elements and symmetric compounds are synthetized by the living cell to form compounds in which the atoms are arranged within the molecule in a specific asymmetric manner. The ability to synthesize must be regarded as a distinguishing property of protoplasm. It is probably fundamental to many phenomena of life. It points to a labile spatial arrangement of the atoms in the molecule constituting a state of dynamic equilibrium. There is probably no change in the configuration of the inorganic molecule, at least there is no evidence of such a change. It is possible that the basic organization in the protoplasm is the result of a certain ability of these groups to shift their atoms and freely modify their molecules in response to the inductive effects of other highly labile asymmetric molecules. Food, external radiant energy, and potential chemical energy, may also induce such shifts. The assumption of such a state of unstable equilibrium might explain why the protoplasm is a thing which is constantly in flux. It might also explain how a stable configuration, which prevents further exchange of energy between molecules, causes the whole to break down into a mass that lacks specific organization and in consequence is merely dead plasm.

Whenever a more stable state is reached in the protoplasm, energy is released and less free energy exists within. Organization in the protoplasm might well be assumed to depend upon the forces exercised

by highly labile, asymmetric molecules, through their asymmetry-including energy upon other molecules.

Such phenomena as resorption, maintenance, growth, enzymatic and hormonal activities, which appear to be fundamentally dependent upon the asymmetry of the bio-molecules, are all manifestations of specificity. This is evident in sperms and ova, but even more so immediately after the fertilization of the ovum when it begins to develop from a particle of organized protoplasm into a very complex being composed of many differentiated and properly assembled cells, tissues, and organs with specialized functions. Some have postulated that electro-magnetic fields, initial directing forces of development, are created by fertilization. It has also been assumed that the germ cells contain a considerable quantity of potential energy which is converted into kinetic energy. This latter form of energy is thought then to be the directing influence which is responsible for the formation of the characteristic unstable asymmetric bio-molecule. This influence is supposed to extend from molecule to molecule much as in a magnetic field, when induction from particle to particle is effected. The imagination can hardly follow the intricacies of the electro-dynamic operations that are conceivably set up by the release of such trigger energy in the fertilized ovum. The possibility of artificially producing monsters tends to indicate that this electro-magnetic field is influenced by the particular environment. The structural organization of the germ cells and the fertilized ovum is conceived as the result of a building up of electronic complexes of sufficient energy to direct the formation of new asymmetric molecules, and so to provide the elements for the synthesis of the specific, characteristic, protoplasmic compound. As a matter of fact the organization of the protoplasm is such that external energy can be bound by it.

The plant builds asymmetric compounds from symmetric material. Some explain this activity by assuming that the necessary energy to accomplish this is furnished by radiant energy. This suggests the idea that external radiation can influence the organization of the plant protoplasm and that the living cell can utilize radiation to attain definite ends. It is not impossible that the protoplasm can release radiant energy as well as absorb it, but the speculations of Gurwitch regarding mitogenetic radiations are generally regarded as questionable and lack the support of satisfactory evidence. In this connection it should be

remembered that the physical characteristics of bioluminescence have not received much attention and that chemical processes involved have not been much explored. It is a fact, however, that many one-celled organisms do emit light. In many cases the mission fulfilled by the emission of light by organisms is biologically fairly well understood. Autogenetic light is less frequently noted in plants than in animals. It is significant that in the plants, in which it is noted, the metabolism resembles the metabolism of animals rather than that of the higher heterotrophic plants.

The kinetic energy which originates from the changed potential energy by some kind of organization in the protoplasm appears in the form of heat, movement of masses, radiation, or electricity. The potential energy which is bound in the protoplasm is stored in the form of such chemical compounds which can be degraded so as to release the energy in the form of kinetic energy. The remarkable thing about the changes in the form of energy in organisms is the controlled manner in which this occurs. In the inanimate objects similar changes usually occur in an all-or-nothing manner, like exploding dynamite. Here the change is complete unless some intelligence intervenes with a device to regulate the change in a purposeful manner. In muscular activity we find a good illustration of the regulation of the change in the form of energy. The amount of energy expended is the amount required. By means of some internal organization the reaction in muscles is discontinued when the result is obtained and no energy is wasted as it is in dynamite explosions. This is accomplished in a purposeful manner by some internal organization.

Another illustration of the purposeful manner in which potential energy is changed into kinetic energy in organisms is furnished by the process of cell enlargement in plants. After the cells are produced in the meristem they enlarge enormously so that the protoplasm appears only as a thin film pressed against the enlarged wall by the cell sap which has greatly increased in volume. In seedlings the necessary kinetic energy has its source in the potential energy bound up in the materials stored in the seed. The process of cell enlargement was at first explained as the result of the hydrostatic pressure in the sap cavity. After the discovery of the phytohormone, auxin, and the induction of this kind of growth by the growth-promoting substance, it was attributed wholly to the active growth of the wall by intus-

susception. Later it was shown that the solutes in the cell sap increase in proportion to the growth; evidently the suction tension of the cell is maintained and the pressure within increases as the cell enlarges and becomes mature. It appears evident that the biochemical regulator, auxin, acts on the protoplasm in such a manner that the cell enlarges with several concomitant manifestations of changes of energy from the potential to the kinetic form. This points very definitely to a nicely balanced organization in the protoplasm for the regulation of the change of the form of energy in a purposeful manner.

Recent experimental results by many investigators make it practically certain that the cell wall is built up by the regular aggregation of giant molecules which are arranged in definite patterns. The giant molecule is more and more being thought of as the unit of structure and of organization in the living protoplasm. The giant molecules may be associated in a purposeful manner to form a connected system. This concept affords a more reasonable explanation of the qualities of various parts of the cell, rather than the notion that the protoplasm merely is a mass of colloidally dispersed particles which are individualized and independent without any relationship to each other. The organization of the plasm within the cell, whether it be generalized as in the one-celled organism, or specialized as in the tissue of the higher organism, does not appear as readily from chemical investigation as from morphological or physiological study. This is so because chemical investigation requires chemical analysis which is not possible without destroying or at least disturbing violently the molecular organization of the protoplasm with chemical reagents. Life and the associated organization of the entire complex ceases as soon as the molecular organization is destroyed. Besides water and inorganic and organic foreign substances required for building the mass of the protoplasm, more specific ones are known to be essential as for example, certain amino acids. A third group of substances appears to be required to regulate the metabolic processes rather than serve as building material. Extremely minute doses are sufficient to this end and larger doses may be toxic or even lethal. Koegl uses the term "oligodynamic factors" to designate them. Growth-promoting substances and chromatin substance, enzymes, vitamins, hormones, belong to this group of biochemical regulators.

E. Enzymes, Vitamins and Hormones

In recent years the attention of biologists has been focused on the activities of three kinds of biochemical regulators that play very important rôles in the organization, namely, the enzymes, vitamins, and hormones. The chemical structure of only a few of these is known but there is sufficient indication that these three classes eventually will come under one head, as for example, biochemical regulator. Three different classes have been made because knowledge of them has been obtained in three independent domains of research. Difficulties of classification are springing up because the distinguishing marks that were thought to be characteristic are now not as clearly defined as they were at first thought to be.

1. *Enzymes*

Enzymes are organic catalysts which play an important rôle in cellular chemistry. They initiate or accelerate chemical changes in other substances by their mere presence. Most of the chemical changes would not occur at all at the normal temperature of protoplasm or would occur at so slow a rate that the dynamic equilibrium would seriously be disturbed, if the enzyme were not present. Some of the activities of the protoplasm have been duplicated in the laboratory but in doing it temperatures and pressures had to be employed which are lethal for protoplasm. In the experiments which mimicked the activity of the protoplasm such quantities were used that are enormous in comparison with those used in the cell. The results obtained in the laboratory are pitifully meagre in comparison with those obtained in nature, no doubt because we lack sufficient knowledge of the organization required to bring about the reactions so quietly and so easily.

The activities of the enzymes represent an integral part of the general organization of the protoplasm. This fact is illustrated clearly by the activity of the glycolytic enzymes called zymases. The necessary energy which is released by the glycolytic enzymatic oxidation of glucose appears to be the special functions of the fine granules in the cytoplasm. If the cells are impaired, that is, if the structure is disturbed, the glycolytic activity is seriously impaired though not necessarily completely destroyed.

The colloidal nature of enzymes prevents their free diffusion

through membranes so that they usually operate within the cell. When they do diffuse they usually operate near the point of diffusion. Cells which produce extra-cellular enzymes usually are specialized and have a glandular appearance.

Extra-cellular enzymatic activity is noted more frequently in animals than it is in plants. The digestive processes illustrate this fact best where the higher animals have a specialized tract for the purpose. In plants extra-cellular digestion is noted in the insectivorous plants and in the germinating seeds of the cereal grains. The rule for plants and protozoa is intracellular digestion.

Some crystals isolated from biological systems and reasonably assumed to be the active enzymatic factor proved to be protein in character. This suggests that the enzymes are an intrinsic part of the organized protoplasm even though its isolation does not always destroy its activity.

In several cases, as for example, the manner in which glucose is transformed into galactose, a spatial rearrangement of the atoms in the molecule is produced by enzymatic activity.

The enzymes are not differentiated by the chemical structure of the crystal, but by their effects on other substances. In the near future this method probably will be discarded. The present method followed from the fact that they manifested specificity. The specificity clearly indicates organization in the protoplasm itself and also that the enzymes form a part of the organization. An effort was made in the nomenclature to indicate the specific substance on which a given enzyme will act. There is a specific enzyme for practically every chemical change that takes place in the organism.

As an illustration of specificity, the activity of the enzyme which will split ordinary sucrose into glucose and fructose may be cited. It will not affect the closely related lactose. In the laboratory glucose can be transformed into fructose but not into galactose. In the human body, however, fructose is transformed easily into galactose. If the need arises it also changes glucose into galactose in the mammary glands where the special enzyme required for the purpose is present. This clearly demonstrates the flexibility in the organization by means of enzymes so that a given end may be attained.

In general it can be stated that the protoplasm produces and contains substances of specific asymmetry which cannot be elaborated in

any non-living system. There can be little doubt that enzymes belong to this class of substances. Their asymmetry is manifested by their power to rotate polarized light. Enzymes frequently are so specific that they differentiate between two isomers in the substances which they affect. The effect on one of them may be slight or completely lacking. Considering the problem of asymmetric synthesis in the protoplasm, directive forces other than those not contained in the enzymes must be postulated, because the optically active enzymes receive their own directive forces and specific structure when they are produced in the protoplasm.

The enzymes are involved in the maintenance of the dynamic equilibrium of the protoplasm. With their aid parts are constantly being broken down and others are synthesized. The balance is maintained within very narrow limits under normal conditions but may be shifted beyond these by pathological changes.

Enzymes catalyse the decomposition of the protoplasmic proteins and catalyse the synthesis of amino acids into new protoplasmic proteins. The "breakdown" of the proteins is well illustrated by the "autolytic" enzymes present within the cells. Their activity can be observed in the test tube. Sterile meat, kept in the incubator at body temperature, decomposes after several days in spite of aseptic conditions. The protein is changed into the constituent amino acids.

Numerous examples can be cited to illustrate the extra-cellular enzymatic activity involved in the organization which exists among the parts of an animal. The circulatory system is one of the very important systems by means of which the functions of the organs are made possible and are coördinated for the satisfactory functioning of the organism as a whole. Clotting of the blood is all important to guard this system when injury threatens discharge of the blood, but thrombosis within the vessels can easily prove fatal. Teleologically the functioning of blood clotting is easily understood. The explanation of the mechanical operations is not so simple. At least three enzymes are involved. When the protein, fibrinogen, is formed and then is changed into fibrin threads by enzymatic activity, a net-work is built up in which the corpuscles become enmeshed to form the clot. The lack of clotting within the vessel under normal conditions is ascribed to the lack of the enzyme, thrombokinase, which is released from the blood platelets only when they are injured or escape from the circulatory system.

Another example to illustrate the importance of enzymes in the general organization is the enzymatic activity by which melanin is formed. The pigmentation of the skin exposed to sunlight is a part of a protective mechanism. The melanin is produced under the influence of oxidase which is contained in the cells. This catalyst induces the oxidation of the chromogen to form melanin.

The preparation of the foods in the alimentary canal of the animal to make resorption possible involves the catalytic activity of many varied enzymes. The carbohydrates, the fats and the proteins all have their specific enzymes which operate only at certain hydrogen ion concentrations. The proper acidity or alkalinity of the food is assured by the organization of the alimentary tract.

The hexoses are practically the only resorbable cleavage products of the carbohydrate foods. The cleavage of these proceeds in graded steps with the aid of specific enzymes. Several different ones split the starch through a number of stages to maltose. Maltose, itself, is broken down by maltase which is present in the intestinal tract. Invertase which splits sucrose into glucose and fructose is also present in the tract. The infant organism readily splits the lactose molecule into the resorbable hexose units but not so the adult organism, since this lacks the necessary enzyme to catalyse. The consequence is that milk sugar acts as a laxative in the adult since the non-resorbable material absorbs much water.

The digestion and resorption of fats is more complex than in the case of the carbohydrates. The pancreatic juice furnishes lipase and the glands of the intestinal wall also elaborate fat-splitting enzymes. The contents of the small intestine normally are slightly alkaline. This is as it should be because the fat-splitting enzymes operate only in an alkaline medium. Unsplit neutral fat which reaches the blood stream is exposed to the action of special lipases.

The mechanism involved in the digestion and resorption of the proteins is even more complex. As in the previous cases, the breakdown occurs in graded steps each resulting in a chemical substance of less molecular size. There are specific enzymes for all stages. Protein is changed to proteose and this to peptones. Peptone is changed to peptids and so on until finally only amino acids remain. About 20 different kinds of these acids, the molecules of which differ in concentrations and combinations, are present in the end-product.

2. *Vitamins*

Minute quantities of certain substances which have their origin in plants are known to be indispensable for the proper functioning of the animal organism. They evidently are essential in the organization of the protoplasm. It is for this reason that some have called them "exogenous hormones." They more commonly are called vitamins. Some animals are capable of synthesizing some vitamins within their own system but the necessary elementary parts are received from plants.

The production of the elementary parts or the entire vitamin itself in plants may be interpreted to mean that radiant energy serves as a directional influence upon the character of the molecular structure of the newly-formed cellular substances.

The vitamins of known structure are asymmetric. Vitamin C, for example, is an asymmetric unsaturated compound and has one double bond in its molecule. It is oxidized readily, hence it is a reducing substance resembling glutathione in its cellular activity. It aids in the re-activation of endo-cellular enzymes.

The fact that vitamins occur in plants in relatively large quantities in the regions of greatest metabolic activity, suggests that their function in the plant at least is concerned with the nutrition and growth. In the animal organism they may play an important rôle in nutrition, resorption, growth, and regeneration. There is good evidence that they also serve to promote respiratory and metabolic processes. It seems probable that they exert their influence on cellular functions by means of the energy set free in the configuration changes of the labile asymmetric vitamin molecules.

Different vitamins affect different tissues. While the activity of the vitamins seems to be a general one, some at least are without doubt specific. The unspecific effects may be the end result of the specific functions. Among the unspecific functions, increased resistance to infection may be cited to illustrate the point. Resistance to infection is claimed for Vitamins A, B, D, and G. Maintenance of the general organization by means of the specific functions of these vitamins would certainly help the organism to resist disease.

Our knowledge of the vitamins and their functions is insufficient to permit a satisfactory conclusion to be drawn regarding the possible relationship that conceivably binds them into a system. Evidence

suggests strongly that such a relationship does actually exist. It is a question whether the effects of an avitaminosis are due entirely to the lack of a particular vitamin or to a disturbed balance that should exist among the vitamins as a whole in the organism.

Apparently there exists a relationship between the vitamins and the hormones. Vitamin A, for example, exerts a regulatory function on the production of thyroxin. It is thought that "anti-sterility vitamin" (E) has an influence on the pituitary gland. The anterior lobe of the gland is rich in Vitamin E.

Chronic avitaminoses are definitely promoted by the abnormal life as it is led in the large modern cities. Some of the faults that are responsible appear to be the lack of ultraviolet light due to the smoke, dust, and grime; to excessive shade; to refinement and paucity of vitamins in the food consumed; to excessive use of alcohol and condiments. These last two induce irritation in the mucosa of the digestive tract and prevent the resorption of the necessary vitamins into the blood stream.

The protective value of vegetables and fruits by vitamins is increased by a greater variety of fruits and vegetables that are eaten and are not over-cooked. It is not safe to focus attention on the consumption of certain vitamins in concentrated form, so long as we are in the dark regarding the importance of the balance of the vitamins among themselves in a given organism. We should know much more about the effects of the concentrates alone and in combination with other vitamins before they are dispensed to the public without the advice of a physician. If the vitamins are taken into the system with a normal diet there seems to be little danger of getting an excessive amount. Nature seems to have arranged for this. Apparently the system does not resorb more than is required to maintain the balance. Any excess seems to be prevented by the cessation of resorption or by active destruction of the vitamins when the limit of the requirements has been reached. Addition of concentrated vitamins to the normal diet does not seem to help to increase the resistance to disease and does not offer unusual protection against illness. If the diet is not normal the addition of vitamin concentrates may eliminate some of the ill effects that usually follow as the result of faulty diet. If the organism fails to absorb sufficient vitamins from a normal diet the difficulty is to be sought in faulty organization that hardly can

be corrected by merely supplying vitamin concentrates in heroic quantities. The injudicious use of concentrates of Vitamins A and D has been shown to be distinctly harmful. The administration of concentrates of Vitamins B and C has thus far not shown any harmful results.

The organism is a carefully balanced unit which is so complex that any administration of potent biochemical regulators should be viewed with suspicion since it may seriously upset the dynamic equilibrium.

3. *Hormones*

Organisms come into existence with the genetic equipment which links them to their ancestors but this does not prevent them from developing acquired characteristics that mark them with a distinct organic individuality. This individuality develops as a function of the organism's reaction to certain factors in its environment. In this sense the body of the mature organism is a recapitulation of its past history. It is true that the thoughts, emotions, habits, and avocation of man are expressed in his physiognomy. The human body is extremely complex and the idea of the association of the many different races of cells, each comprising billions of cells, is stupendous. All through the body these cells are kept in constant communication by means of a wonderful general organization. Not only are the communities and the individual cells supplied with the necessary nourishment and freed from the waste products of their metabolism, but also they are stimulated to activity or are restricted in their activity as the general need of the organism requires. These activities are regulated by the nervous system and biochemical regulators called hormones. These hormones are elaborated in specialized glands that are without ducts and are called endocrine glands.

Our knowledge of these mysterious substances developed from what we learned of the physiology of the glands, and the effects of the substances produced by them. They were classified according to their activities, and not according to their chemical structure. It is probable that in time the biochemical regulators, enzymes, vitamins, and hormones will not be classified according to the many marks by which they now are known but rather according to their chemical constitution. Even now certain of these regulators can be classified simultaneously as enzymes and hormones even though they are known

to have but one definite chemical structure. Our knowledge at this time is insufficient to begin a reform in this domain.

The hormones exercise a very profound influence on the metabolism of the specific cells that they control. The adaptation of the organism to its given environment is largely the result of the activity of the hormones. The nervous system exercises control over the glands, which secrete the hormones, so that emotional states influence the activities of many cell communities and cell races via the endocrine glands. It is in this manner that the mature organism has become a recapitulation of its past history as expressed in the sum of its acquired characteristics. In the human being the physiognomy is particularly expressive of the individual's past history.

The animal organism has the peculiar property of building its own body from new compounds produced by itself. The blood supplies these materials to the cell communities, and in addition it supplies such materials as are required to furnish the necessary energy and those master regulators, the hormones, which dictate growth and the liberation of energy.

Examples of the glands that exercise such a profound influence on the body are the thyroids and the suprarenal glands. They synthesize from the organic medium, the new and extremely potent thyroxin and adrenalin. These influence not only the cells and organs but also the mental activities. This fact clearly demonstrates that the organization observed in organisms is on a much higher level than the organization that is sometimes ascribed to inorganic things. Not only do the various parts coöperate in a regulated manner, as in a machine, but the regulators themselves which produce the stimulants or inhibitors are produced by the organism itself. It produces these regulators as the need arises to promote the welfare of the individual or to insure the preservation of the species.

The hormones are chemical substances that are elaborated by living cells and are distributed by the blood and lymph to all the cells of the body where they exert specific influences on the chemical changes taking place there. They are extremely potent. Incredibly small quantities produce remarkably great results. Their stimulation produces activities involving a considerable amount of energy. An excessive quantity can produce serious injury by over-stimulation.

Since the hormones are produced in ductless glands from which

they diffuse into the blood stream they also have been called "internal secretions," "incretions," and "endocrine secretions." The materials from which the hormones originate are stored in the glandular tissue. Production goes on uninterruptedly but delivery to the various sites of activity is regulated and often is intermittent.

The endocrine glands are richly supplied with blood vessels and the capillaries are wide and sinus-like which is indicative of the preparedness of the system to carry unusually heavy loads upon demand. This is well-illustrated in the case of the adrenal glands where besides an abundant supply of blood vessels the musculature of the veins differs from that of other veins. The adrenal veins contract to such an extent that they cause retention of the blood in the glands which becomes highly charged with hormones and upon being stimulated the glands quickly discharge the blood into the circulatory system.

The thyroids, parathyroids, adrenals, and pituitary glands are permanent glands and have purely endocrine functions. The pancreas and the gonads fulfill a double duty; that of carrying on their specific function and that of producing hormones. The pineal body and the thymus are temporary endocrine glands that function only during the pre-puberty period of life.

The endocrine glands are united into a system which is closely associated with the nervous system. These two systems mutually stimulate each other. The nerve centers, the autonomic nerve fibers, the endocrine glands with their specific hormones constitute a complex maze, in which it is often difficult to distinguish the simple relationships. This fact alone should deter anyone from risking disturbance of the equilibrium of so finely a balanced organization. The chemical and nervous regulatory systems are still more closely allied in that the transmission of nerve impulses involves the liberation of chemicals such as acetyl choline and sympathin.

The hormone balance can be interfered with by the introduction of a foreign element into the blood stream. A simple illustration of such intervention is the smoking of tobacco. It causes increased epinephrine secretion. This causes an increase of glucose in the blood. The adrenal medulla has responded by throwing epinephrine into the blood stream and the epinephrine causes the glycogen of the liver to be converted into glucose which promptly enters the blood stream. The "lift" which is experienced from the cigarette partly is due to the

raised glucose level of the blood and partly to the direct stimulation produced by the epinephrine itself. The contraction of the arterioles due to an excess of epinephrine causes habitual smokers to have subnormal skin temperature in the hands and feet. Not only is the hormone of one endocrine gland influenced by the hormone of another gland but also by vitamins that are ingested and resorbed.

The master gland of the endocrine system is the pituitary gland. An impressive list of hormones has been extracted from this gland. The anterior lobe elaborates at least nine different hormones: a growth hormone; a gonadotropic hormone; a thyrotropic hormone; a hormone which affects the fat metabolism; prolactin which controls the milk production in nursing mothers; hormones which activate the growth and the function of the adrenal glands, the parathyroids, and the islet tissue of the pancreas respectively. The interrelationship between the gonads and the thyroids, adrenals and the pituitary gland has been verified experimentally.

The marked relationship that exists between the endocrine system and the nervous system has been verified experimentally and is suggested by the anatomical relationships of the two systems of organs. This is evident particularly by the innervation of each of the endocrine glands. The nerve impulse arrives at the gland and provokes a change in the colloidal state of the cytoplasm so that it becomes less viscous. Some of the water which previously was "bound water" becomes "free water" after stimulation, and since it again manifests the ordinary physical qualities of water, it becomes a carrier of the active solutes that diffuse into the blood stream. This power of the endocrine tissue to change the viscosity of the cytoplasm under stimulation from the nervous system is characteristic.

Hormones are often stored in the glands as complex combinations with other substances. The nervous impulse in some way liberates the active principle from the combination and the hormone is resorbed into the blood. The structure of the thyroid offers a good illustration of the manner in which an adequate arrangement of parts is reached by the organization to meet a given need. It receives nervous impulses via the sympathetic nerve fibers. The active tissue of the thyroid parenchyma contains small vesicles which are filled with a colloidal substance and the stored secretions. The vesicles are separated by epithelial and connective tissue in which the blood

vessels, the nerve fibers, and the lymphatics are located. The thyroid gland is richly supplied with blood vessels and lymphatics. The structure of the thyroid gland varies from time to time and in general is very labile, adapting itself to the particular needs of a given time as for example changes in temperature in different seasons.

One of the characteristics of hormones is that they act at a considerable distance from the point of their origin. The blood stream is the medium of translocation. Apparently some potentially active combination of hormone and some foreign substances exists in the blood stream and the active principle is liberated only at the site of activity. The site of activity is probably made receptive for the active principle by nervous stimulation. The complicated function of endocrine glands and their specific hormone may be illustrated by the thyroid gland and its hormone, thyroxin, and again by the adrenal glands and their hormones.

Thyroxin, the hormone elaborated by the thyroid, is related to the oxidative process in all of the cells. About one milligram is resorbed into the blood stream daily. This is sufficient to induce oxidation in the countless cells of the body. An extra milligram injected into the blood of a normal individual produces hyperthyroidism and greatly increases the oxidation. This is indicated by the greatly increased amount of carbon dioxide eliminated. The water metabolism and the salt metabolism also are affected either directly or indirectly so that more water and salt are excreted in the urine after the added stimulation.

If insufficient thyroxin (hypothyroidism) is produced during the period of growth, the maturation process is retarded. The fat which is not metabolized under these conditions is deposited, the generative organs do not develop, the skin remains dry and thin. The nervous system is also affected by thyroid hypo-functioning as is manifested by lack of alertness, apathy, and general weakness. In the adult thyroid deficiency also produces a tendency to obesity, sexual weakness, and general apathy.

Excessive thyroid activity is manifested by loss of weight, extreme nervousness, palpitation, tremors, excessive perspiration, light mindedness, gastro-intestinal disturbances, and sexual over-stimulation and a general restraint of the vital processes. The active principle has been isolated and was found to be laevo-rotatory.

Thyroxin is built from iodo-tyrosine and di-iodotyrosine which are elaborated in the thyroid gland. The diet usually contains sufficient tyrosine but in certain localities, the diet may be deficient in iodine. Apparently the response of the thyroid is in the form of an effort to overcome the deficiency by increase of the capacity of the gland and thus increase of the output by enlarging the gland. The enlargement of the gland may be merely caused by an increase in the amount of colloidal substance that is stored. The formation of this pathological enlargement is often prevented by addition of iodine to the diet. Over-dosage of iodine may have harmful results because it may induce the production of an excess of thyroxin which produces toxicity.

In the case of malfunctioning of hormones in general and thyroxin in particular, the cause may lie in the concentration of the active principle in active form rather than in the kind of hormone present. Sometimes the lack of the proper concentration may be due to over-production or under-production but it may also be due to the formation of racemic mixture or dextro-rotatory material instead of laevo-rotatory material. Thus the hormone fails to stimulate because there is not enough or too much material of the proper stereo-structure.

The mechanism of operation of hormones may be illustrated by the manner in which thyroxin arrives at the site of activity. The thyroid produces a protein, thyroglobin. From this the active principle, thyroxin, may be split off and be secreted directly into the blood or it may be circulated as a member of a trinity of amino acids which split only upon arrival at the site of activity by nervous stimulation or proper receptivity of the cells or the tissue in a given location.

The effects produced by the hormones of the adrenal or suprarenal glands also furnish a good illustration of the high degree of regulation exercised by the hormones and the importance of these biochemical regulators in the general organization.

The suprarenal glands are located upon the upper pole of the kidneys. They are small bean-shaped bodies weighing about one-fourth of an ounce. They are composed of an inner medulla and an enveloping tissue called the cortex. A thin layer of cells separates the medulla and the cortex. The medulla stands anatomically in close relationship with the sympathetic division of the autonomic nervous system. The cortical portion of the gland consists of three layers of cells which are rich in sterols and lipids. The blood supply is abundant. The

special musculature of the veins of these glands assure the thorough mixing of the active principle in the blood and the rapid distribution of the mixture in the general circulation. The particular function of the active principle demands that it be well mixed and that it reach the site of activity with great speed. While the medulla is under the influence of the sympathetic nervous system, the cortex is regulated by the parasympathetic system. The two regions of this gland secrete different kinds of hormones. The medulla secretes epinephrine and the cortex cortin. Both of these hormones are of the greatest importance in the organization.

Epinephrine has been isolated in crystalline form and its structure has been determined. It also has been synthesized. The active form is laevo-rotatory. The extract and the synthetic product are in racemic mixture and are not optically active. The dextro-form is not physiologically active so that in racemic mixture a double dose is necessary to produce a given result. Both thyroxin and epinephrine are related to the amino acid, tyrosine, and may be derived from it.

The active laevo-form of epinephrine is sensitive to alkalinity and becomes inactive if the pH rises beyond 7.6. Calcium and cholesterol tend to increase its activity but potassium and phospholipids such as lecithin, for example, tend to decrease the activity. The sensitivity of this hormone to the alkaline state illustrates the importance of having the medium of translocation in the proper state to insure the proper physiological activity. The secretion of epinephrine apparently is continuous and it seems to enter the blood combined with a lipid. This suspends its physiological activity until it reaches the site where its functions are to be exercised.

The liberation of significant amounts of epinephrine takes place only when the organism must cope with some emergency. In an organism under stress, the nervous system stimulates the gland to secrete the epinephrine which s quickly circulated so that the physiological effect is noted almost instantaneously. Exposure to severe cold, decreased oxygen supply, intense pain and emotional upset, such as fright, terror and rage, evoke an increase in the activity of the medulla of the adrenal glands with consequent physiological effects.

Epinephrine causes contraction in the blood vessels of the skin and the viscera but strangely enough it dilates the vessels of the heart, the coronary arteries, and the skeletal muscles. As a result the blood

is quickly shifted from the skin and the viscera to the organs where increased oxidation and energy release are in demand to cope with the emergency. The blood pressure, the pulse rate and the respiratory rate rise simultaneously with the injection of the active principle into the blood stream. Epinephrine by stimulating the sympathetic nervous system causes the breakdown of the glycogen stored in the liver from which glucose results that is immediately furnished to the blood and is carried by it to the regions where added fuel supply is required. When the glucose level drops in the blood at any time the adrenal medulla responds by increasing its secretion with the consequence that the glucose level is restored. The adrenal medulla affects the general metabolism as well as the carbohydrate metabolism. This influence probably is exercised in conjunction with the activity of the thyroids which are stimulated by the rapid consumption of the oxygen during periods of stress.

Excess of epinephrine in the blood causes "flushing," shift of blood from the viscera to the heart, lungs, muscles, and the central nervous system. Adrenal insufficiency causes undernourishment and low blood pressure, gastro-intestinal complaints, vague pains, feelings of distress, and nervousness.

The active principle secreted by the cortical portion of the adrenals also has been isolated in crystalline form and has been called cortin. Its function is maintenance of the volume of fluid in the circulatory system. It prevents muscular fatigue and assists recovery from fatigue after periods of stress. The cortical region of the adrenals is one of the first points in the organization where reaction is noted when infectious bacteria invade the organism. Cortin in some way promotes the organism's resistance to infection. Extended illness can weaken the secretion of this gland to the point where recovery is slow. Thus, it is with the feeling of weakness which sometimes persists a long time even after recovery from an extended mild infection. Such infections have impoverished the cortical secretions of the adrenal glands.

Cortin has a decided influence on the gonads. This may be an indirect influence involving the activity of the pituitary gland. It is certain that the three glands coöperate very closely.

The proper functioning of the endocrine system depends upon physico-chemical and on biological factors. It depends, first, on the

production of the optically active principles in the proper concentrations; secondly, upon the nerve impulses and the response to these impulses by the glands so that the active principles may be formed and enter the blood either in the free state or combined with other materials; thirdly, on the nature of the medium of transportation by means of which the active principle is to reach the site of activity, slight changes in the nature of the medium preventing the active principle from reaching this site in the physiologically active form; fourthly, on the response of the tissue which is to be affected—if the activity which is to be induced is of a metabolic nature, then the stereo-configuration of the hormone and of the protoplasm to be affected must be of specific structure; and fifthly, on the balanced state of the different hormones that are carried in the blood stream at a given time.

Malfunctioning of the endocrine system occurs when the above five required conditions do not obtain.

It is well to keep these possibilities in mind in seeking the cause of diabetes, since the mechanics of the activity of insulin are not known. It is assumed that under the influence of the insulin, the glucose in the blood suffers some non-oxidative change which enables the cells to absorb it. The change effected may be one of stereo-configuration. Insulin has been found to be very widely distributed in the tissues of some cadavers of diabetic animals. This suggests that in some cases at least the disease may not be due to a lack of the production of the hormone, insulin, but to failure at some point in the organization which makes the proper functioning of the insulin impossible.

Some substances that induce certain activities simulative of hormones, are called parahormones. Most of these are waste products of metabolism. The most striking example is carbon dioxide which stimulates respiration. It influences the respiratory center in the medulla. The rate of the impulses delivered to the muscles that cause the breathing movements is determined by the concentration of the carbon dioxide in the blood. Higher concentration induces more rapid breathing and increases the circulation of the blood. Increased ventilation in the lungs reduces the breathing rate. This influence is not as specific as that of a true hormone; it is the pH which determines the rate. It nevertheless is another good example of the fine and

automic regulation of organization. When the lungs have eliminated the carbon dioxide, the blood still contains the non-volatile acid waste products which must be eliminated in the kidneys. The increased circulation also speeds up the elimination of these waste products of metabolism. The functioning of the lungs and kidneys is of prodigious efficiency. It is the activity of these organs that makes the compactness, agility, and high rate of metabolism of the human body possible. Without them the other arrangements to supply the countless cells with nutrients and to eliminate their waste products would be useless.

F. Biochemical Regulation in Ontogenetic Development

The organization of the protoplasm enables it to maintain itself and to increase its mass. The increment of its mass and volume is the fundamental notion of growth. The increment of the volume introduces a problem of maintenance because the increment of the area is not proportional to the increment in volume. The ratio of the area to the volume constantly grows less as the volume increases. The increment of the volume of protoplasm is followed by an equivalent increment of the total metabolic activity, which demands increment in facilities to supply the necessary metabolites and to eliminate the consequent waste products. The exchange of material between the protoplasm and the environment is limited by the area which delimits the protoplasm. Evidently with the constantly decreasing ratio as the protoplasm grows in volume, a point is reached at which the area is no longer sufficient to meet the requirements and the protoplasm is threatened with death unless the optimum ratio between the area and the volume is restored. Death is prevented by cell division. During the growth and the development of an organism the cells constantly divide and the optimum ratio is restored. This is the fundamental notion of growth by cell proliferation. This kind of growth is controlled by biochemical regulators. Some of them stimulate the division and others inhibit it. There is a third kind of growth, common in plants, exceptionally present in animals, namely, growth by cell enlargement. In this kind of growth, the cells that are proliferated in the meristem grow to maturity by increasing the mass of the cell wall and the contents of the cell sap cavity

but not materially increasing the mass of the protoplasm. This kind of growth also is controlled by growth substances.

The unfertilized egg in plants and animals is undifferentiated and non-specialized. After fertilization, however, proliferation and differentiation begin almost immediately. Morphologically like cells assemble and form groups in a purposeful manner, to form tissues and organs that have specific functions. Apparently this process also is under the control of growth substances. By an evident organization the development and well being of the organism and the preservation of the species are assured.

The egg can be induced to proliferate without normal fertilization. In order to do this it is necessary to disturb the equilibrium of the protoplasm which keeps the egg in suspended animation. This fact that a disturbance of the equilibrium which keeps it in suspended animation is required, indicates that the fertilizing sperm cell achieves a similar result by introducing a biochemical regulator. Ths probably is the main function of the chromatic substance with its nucleoproteins that is introduced by the sperm. The stimulation of the metabolism of the egg is evident immediately after fertilization. Either the sperm has added a substance that stimulates the metabolism or introduced an inhibitor to a substance that suspended animation in the egg or both accelerator and inhibitor were added. Efforts to extract such substance from the sperm have thus far failed. It is certain however, that proteins and lipids in the egg, which remained undisturbed before fertilization were immediately digested after fertilization. The enzyme that is responsible for this digestion may have been in the egg or in the sperm. It seems probable that it was in the egg but was prevented from exercising its activity until the sperm introduced some substance which activated it.

The initiation of growth by fertilization suggests the introduction of biochemical regulators that continue to be active throughout the life of the organism. The influence of such biochemical regulators need not be limited to the confines of a single cell. In the developing embryo definite centers of influence have been noted. The influence is produced either by the diffusion of chemicals or by some inductive method possibly by radiant energy. Such induction would explain

the effect produced by the "organizers" of Spemann and the "evocators" of Waddington and the "auxins" of Koegl.[1]

The process of cell differentiation in which the cells become specialized and their function becomes restricted probably has the same mechanism in plants and animals. The organizers, evocators, and auxins must have their origin in the protoplasm. In a series of steps the mass of the protoplasm is increased; is divided and differentiated and the potencies of different regions become more and more restricted. For each area in the embryo, there comes sooner or later a time in the ontogenetic development when its future is finally determined.

In the amphibian gastrula any part of the ectoderm can be differentiated into a neurol tube. The "organizer" is made responsible for the fact that only one neurol tube is actually formed. Certain substances have been isolated which have the power of initiating the differentiation of the ectodermal cells to form the neurol tube. The chemical nature of this substance is not highly specific because similar results can be obtained with highly purified oleic acid and several other organic acids. Saturated acids including stearic acids fail to induce such effects.

While these results indicate that the agents are of a chemical nature they do not give a complete picture of the organization because regulation as well as stimulation is noted in the development of the embryo. Inhibition of differentiation as well as stimulation to cause differentiation are necessary so that only one neurol tube develops among all the equally potential ectodermal cells.

Experimental evidence indicated that the process of pattern formation in the embryo, is induced by evocator substances, diffusing or radiating from larger to smaller areas already formed in a definite direction. The type of pattern which is formed depends upon the nature of the evocator, the area affected, and upon the balance in the entire system. Without doubt the chromatin substance of both the egg and the sperm cell are involved in the mechanism of this organization.

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[1]Spemann's organizer is a chemical substance, probably of the sterol type, in the gastrule which can organize primitive cells to develop according to the development pattern of its source. An evocator is an organizer hormone present in the egg, liberated at the "organizer center" (dorsal lip of the blastopore). It comes from an inactive precursor. It is essentially the chemical stimulus for neuralization. Auxin is a phytohormone which induces growth by cell enlargement.

The physico-chemical process of the pattern formation in embryos when evocation is given at a definite time, in a definite place, under required conditions in the whole system, presupposes a nicely regulated organization in the protoplasm. This organization cannot be explained by our limited knowledge of physics and chemistry. Our limitations should not, however, deter us from attempting to formulate explanations in the light of the latest theories regarding the nature of matter and energy. These theories do not fully satisfy the mind but they do suggest a better interpretation of the manifestations of the organization in the protoplasm.

The nucleo-proteins contained in the chromosomes, which are held to be responsible for the structural characteristics of the body, may be conceived as being composed of molecules having different stereo-configuration and as having the power of perpetuating their peculiar asymmetric structure, and in this manner perpetuating the directing force in the newly formed protoplasm.

The "primum mobile" for the control of the ontogenetic development of the organism would thus be sought in the complex association of the electro-dynamic fields resulting from the symmetry of the electronic complexes, the bio-molecules. The great influence which the sperm exercises upon the ovum may in this way be explained as the propagation of physico-chemical inductions from molecule to molecule which were introduced with the bio-molecules of the sperm cell.

The constant decline of the synthetic processes in an aging organism may be interpreted as an expression of entropy since there is a constant decrease in potential energy which is required for the directing influence and for the generation of new cells to replace injured or aged cells. The trigger energy is degraded in proportion to the number of cells that are produced in the proliferation process. The accumulation of worn out cells represents an increment of the bound energy that is proportional to the intensity of the degeneration process in the senile organism. Conceivably the initial energy that releases the trigger energy itself, is constantly decreasing as the capacity of the bound energy increases; the trigger energy thus becoming less and less. Finally, the potential difference is too slight to permit the formation of new cells at the rate which is sufficient for the organism to maintain itself. Senility and death can be understood in this decline of the effective energy of the organization.

In the plant the organization is manifest in the processes of cell proliferation, cell differentiation, and cell enlargement. As in animals the ontogenetic development does not go on in a haphazard manner but in a regulated manner. A number of biochemical regulators have been isolated that can be summed up under the terms "auxins" and "heteroauxins." In the plants as well as in the animals both accelerators and inhibitors take part in the organization.

Auxins are not as specific as they were first thought to be as is evident from the effects produced with the heteroauxins. The abnormal growth, called galls, which are produced by substances injected into plants by certain insects also induce growths which, however, are more specific for the insect than they are for the plants. The insects that are difficult to differentiate morphologically are easily distinguished by the kinds of galls they produce. Galls morphologically alike are produced by insects of a given kind on different kinds of plants, and different insects will produce different kinds of galls on one and the same plant. A very small quantity of the substance which the insect injects is sufficient to induce a considerable amount of growth and cause the formation of a gall of relatively large size. Thus we see there are cases in which the organization of the plant can be interfered with to the extent that growth has been induced by a foreign biochemical regulator that is more powerful and more specific than the phytohormone.

In a similar manner the process of fertilization of the flower with pollen can introduce a chemical regulator that temporarily at least interferes with the general organization. There exists an antagonism between the vegetative growth and the reproduction in all kinds of plants. In the grasses it is noted that as soon as fertilization is complete, rapid senile changes occur in the vegetative structures. This antagonism is particularly impressive when we consider the manner in which the plants transfer food supply to the reproductive organs during the time of seed formation. The plant apparently sacrifices its vegetative interests and directs its major activities to the preservation of the species. The mechanism by which the transport and storage of the food occurs is not well understood but teleologically it is not difficult to explain that there is definite advantage to the storage of the food in the form that it occurs and that the best interests of the embryo are served and that the propagation of the species is made possible.

G. Organization and Disease

A complex organism like man begins its existence in the fertilized ovum and develops cells, tissues, and organs that are associated in a smoothly operating whole. The cells live in a fluid medium which is manufactured by the organs. This fluid carries the substance derived from the ingested food and the metabolic wastes which are to be eliminated. Chemical Regulators are active throughout this complex system of cells immersed in fluid. The nervous system exercises the greatest influence in the regulation of all of the activities in the complex. The coördinated cells in toto form a perfectly integrated being.

Science has so concentrated on the independent study of the great structural heterogeneity that its composition of many disparate elements, such as liver, heart, spleen, kidneys, pancreas, etc., has distracted the attention from being given to the organization as a whole. The functions are less precisely located than the organs. The prime function of the skeletal structure is support but it also is involved in the nutritional, respiratory, and circulatory systems. It must not be forgotten either that the bone marrow is important in the manufacture of leucocytes and red cells of the blood. If the protoplasm divided and the cells became differentiated and formed organs and arranged them in compact form, it was in order to make it possible for the organism to live more efficiently in its natural environment, allow a high degree of metabolism and still occupy a minimum of space. From the egg to the mature organism the being always is a distinct entity in spite of its increasing complexity.

Throughout the life of the organism the organization of the protoplasm is bound up with the constitution, the arrangement, and the regulation of the independent parts and the interdependent functional activities. When the dynamic equilibrium is undisturbed great silence reigns; there is no feeling or hearing the harmonious workings except a general feeling of well-being called euphoria.

If the equilibrium is disturbed, the euphoria changes to a feeling of malaise, nervousness, general feeling of distress or fear, or of weakness, apathy, or definite localized pain. These are all indications of functional or structural disorders. When people do not feel well, even though they are not distinctly sick they must be considered as not healthy for the organization is certainly not properly balanced. The secretions of some gland or mucosa may be insufficient or too

abundant; some irritant substance may cause an exaggerated excitability of the nervous system; organic functions may not be exactly correlated in time or location; or the organism may not be able to adapt itself properly to the new conditions imposed by infectious organisms.

Disease may be defined as a functional or structural disorder within the general organization. Its aspects are as numerous as the organic activities. In illness as in health, however, the organism preserves the same unity. When it is sick it is sick all over. To understand any illness, it is necessary to realize that there is a disturbance in the general dynamic equilibrium. It is necessary to do all that is possible to determine the cause and not to confound an effect or concomitant factor with the cause. This sometimes becomes very difficult. Once the cause is established, ways and means must be found to reëstablish equilibrium. It is not always safe to intervene violently in attempting to do this and not allow the powers of adaptation to correct the unbalanced state. Sometimes, better feeding, proper exercise, irradiation, supplying sufficient vitamins, etc., can do more than extraordinary intervention.

If the power of adaptation is at low ebb then the disease is particularly dangerous. This is another way of saying that the power of resistance is low or that the constitution is weak. In resisting disease it must be remembered that matter and energy are used. This means that there is a limit to resistance. Unless the balance in the organization is reëstablished before complete fatigue sets in, the organism must die.

Pain is an important indicator of disorder in the organization but intensity of pain is not a sufficient indication of seriousness of the disorder. The nervous system may be too fatigued to register when the organism is dying. The intensity of pain experienced depends largely on the individual susceptibility to pain. It varies according to the sensitiveness of the individual.

There are two major causes of disease, namely, invasion by some disturbing element as viruses, microbes, etc., and progressive disorganization which may be the consequence of the toxins produced by invading organisms or toxins that are produced by the organism's own tissues and are not properly eliminated.

Virus disease is caused by a filterable infectious agent, usually

smaller than bacteria but larger than many other large protein molecules. The virus enters the living cells of the susceptible host and there produces more of its own kind of virus. This increment of mass occurs only in living protoplasm and is sometimes accompanied by the formation of new strains of viruses. When deprived of protoplasm, viruses may survive a long time in the inert state. Some of these viruses have been isolated in crystalline form. Viruses show striking similarities to the genes, for example, in size, reproduction in protoplasm, presence of nucleic acid, mutability, and the production of phenotypic effects. The viruses may hold the key to genetic changes in the living cells and possibly to the origin of the mysterious disease of cancer. If the normal chromatin substance of the cell is the directive influence for proliferation and differentiation, it is not difficult to see how the viruses which are of a nucleo-protein nature can seriously disturb the dynamic equilibrium of the normal cell. They preferably attack the cells of the nervous tissue, of the skin and of the glands. They either destroy the tissue or alter its function. Infantile paralysis, grippe, encephalitis lethargica, measles, typhus, yellow fever, and perhaps cancer are caused by such agents. The organization once invaded by viruses appears to be helpless against their attack.

Bacteria cause disease by entering the organism through the mucosae of the intestine, the nose, the eyes, the throat or through a wound in the skin. They do not enter the cell like the viruses but remain around the cell. They multiply under the skin, between the muscles, in the abdominal cavity, in the membranes enveloping the central nervous system and in the blood. They secrete toxins which can throw the whole organization into confusion and disturb the dynamic equilibrium.

Degenerative diseases, such as arteriosclerosis, myocarditis, nephritis, diabetes, cancer, alimentary deficiency may be caused by toxins of invading organisms, or from toxins produced in the system. Exophthalmic goitre is caused either by a superabundance of thyroxin or poisons produced in the thyroid. Other degenerative diseases are due to a lack of indispensable secretions. Myxedema, diabetes, pernicious anemia are in this class. Still other deficiency diseases are due to the lack of elements required for the construction and maintenance of tissues such as vitamins, mineral salts, iodine, and metals.

The symptoms of disease usually are the adaptive responses of the

organism in an effort to reëstablish the dynamic equilibrium after it has been disturbed. The organic functions are modified to combat the adverse factor. As in all adaptation, the adaptive responses have definite ends in view. They tend to oppose the invasion, to repair the lesions, to the invading organism or to neutralize the poisons produced by the invader or by its own malfunctioning tissues. In many cases the adaptive responses are known to be due to definite chemical substances. When certain polysaccharides, present in the bodies of bacteria, are united with a protein they determine specific reactions in the tissues. The tissues manufacture some carbohydrates and lipoids which have corresponding properties. They give the organism the power to attack foreign proteins or foreign organisms. The organism has the power of producing antibodies that finally destroy the invader. It is in this way that the vaccination and the use of therapeutic serums have aided in this combat of the organism against disease. Artificial immunity can be produced against a specific disease in this manner.

If the patient can command enough forces, he successfully fights disease, either alone or with the aid of serums and of non-specific chemicals and physical therapy and casts out the invading microbes and neutralizes the viruses and toxins. Profound changes take place of which the patient is aware. It is surprising how he often "feels" if he is winning or losing in the fight he makes. Fever, delirium, and acceleration of chemical changes occur. Sometimes lesions develop in various organs, as for example, in the heart, liver, lungs, or skin. At once certain potential powers actualize and combat the disease. The leucocytes multiply, metamorphose according to need, secrete the specific substances that are required, adapt themselves generally to the changing conditions brought about by the pathogenic factors which are: lack of efficiency in the organs; presence of bacteria or viruses; and the accumulation of offensive agents. When abscesses are formed the ferments in the abscess digest the microbes. Living tissues also are broken down to create a channel by means of which the pus may be eliminated. When the organism loses the power to adapt itself and eliminate the disturbance of equilibrium, it is in immediate danger of death.

There are some invaders that the human system cannot adequately cope with unaided. Treponema pallidum which causes syphilis can-

not be killed or eliminated and never leaves the body spontaneously. Cancer also appears to meet with little or no opposition. Both benign and malignant tumors are so much like the normal tissue that no adaptation is evoked. Since there is no reaction, there are no symptoms until the disease has made considerable progress, and then it is that the secondary effects produced by the tumors give indication of the disease.

The degree of resistance which is offered to disease which the human system can oppose by adaptation is largely a matter of individual constitution. In many cases the individual possesses a natural immunity. The nature of this immunity is not clearly understood but probably both hereditary and acquired characteristics are involved. Acquired immunity may be spontaneous, following an attack or it may be induced artificially. Pertussis, typhoid fever, small pox, and measles, for example, render their victims immune, at least for a long time.

Reserve force in a biological system is due to three factors: the stored material, the potential energy, and the general organization. Both rest and activity take place constantly in the human body, in an organized manner, but no tissue is equally active in all parts of the body at all times. The rest and activity of each tissue are repeated periodically with a definite rhythm. Some quiescent cells are recuperating for future activity so that the body possesses a reserve force at any time if the activity of the person is normal. This is not true if the stimulation and drain are excessive. The molecules of fatigued cells have lost their spatial configuration and require nourishment, elimination of waste, and restoration of the proper configuration during a period of rest. Then they become functionally active again. If sufficient rest is refused them the cells cannot fulfill their proper functions. Fatigue is a clear indication that a tissue cannot fulfill its function adequately. When the muscular system is fatigued rest readily restores the cells to fitness. But when the nervous tissue becomes fatigued, the restoration is much more difficult and takes a much longer time. When it is recalled that the nervous system occupies a commanding position in the general organization, it is easy to see how dangerous it is to fatigue this system. If the human body is to resist disease, evidently all of the tissues should be in fit condition, that is, well supplied wth nutrients, cleared of waste products and not exhausted.

Much attention has been given to combating infectious diseases by artificial methods and great successes have been recorded against such diseases. Not much progress has been made in diminishing the ravages caused by chronic affections especially by cancer, diabetes and heart disease, chronic nephritis, brain tumors, arteriosclerosis, syphilis, cerebral hemorrhage, and hypertension. Functional disorders brought on by excess of food ingestion and lack of proper physical exercise have increased. In our city life practically everyone suffers in the course of urban life some kind of attack of neurasthenia which is engendered by worry, constant agitation, and nervous strain.

Apparently in city dwellers the constitution of the average human today is less robust. Men seem to be more susceptible to degenerative diseases and succumb more readily. The lowered resistance may be due to the urban modes of life. Men are subjected more to nervous and mental shocks. More toxins are produced by the fatigued tissues than can be eliminated in the short periods of rest. The food consumed often lacks the elements required to restore the fatigued tissue. The resorption of such elements may not be sufficient even when they are ingested. The atmosphere is not always the best to insure the proper respiration of the tissues.

Mass production in some cases has modified the composition of food because the organization of the domesticated plants and animals has been seriously interfered with. Cereals, fruits, vegetables, dairy products, eggs, poultry and beef, may have retained their appearance but their vitamin products and possibly many other important factors may not be present in sufficient quantity. The crowded condition, lack of exercise, and irradiation of cows, cattle, and hens have modified the foods derived from these sources. Modern urban life does not permit the ordinary citizen to develop a robust constitution that offers protection against degenerative diseases.

Medicine needs to give attention to our modern ills and to the organization of the protoplasm of the individual cell, to tissues, and to organs. The human body as a whole must be better understood, if the modern mode of life is to survive.

Growth, 1944, **8**, 311-319.

NATAL TESTIS WEIGHT AND EARLY POST-NATAL BODY GROWTH IN THE DOMESTIC FOWL

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(Received for publication on September 30, 1944)

Extreme variability in testis size was observed during routine verification of sex in newly hatched chicks. Actual weight of both testes has been found to vary from 2.0 to 22.7 mg. In early observations varietal strains appeared to have characteristic testis sizes. The largest were observed in strains known to have the most rapid growth rate in the early post natal period. No previous reports on the significance of the variability in natal testis size have been found in the literature.

Recently Munro, *et al* (1943) have observed that, at 17 days after hatching, testis weight was relatively the greatest in White Leghorns. In decreasing relative testis weight were New Hampshires, White Wyandottes, White Plymouth Rocks, and Light Sussex. These same authors make the statement that the testes of the White Plymouth Rocks were normally one-third smaller than those of the White Leghorns at 17 days of age.

It is well known that early post-natal growth of the body varies with season of the year and heredity. Jaap and Morris (1937) reported that, in the strains they studied, body weight of White Plymouth Rocks was smaller at eight weeks of age than that of White Leghorns. The latter have a much smaller adult body size. As a result, it was concluded that growth rate to eight weeks of age appeared to be a separate entity and was not necessarily related to adult size. When a small bodied variety grows more rapidly in the early post-natal period a growth stimulus associated with early sexual development probably exercises a greater influence than adult size. The acceleration of early growth in early sexual maturing females has been proved by Waters (1937). While the first ovulation (age at first egg) is a distinct measure of earliness of sexual maturity in females, no definite external endpoint of sexual maturity is avail-

able for males. In poultry practice it is well known that external manifestations of sexual activity appear much earlier in strains of the same variety which have a more rapid early growth.

Natal Testis Weight and Post-Natal Body Growth

Four varietal strains were chosen to obtain definite records on the relation between testis weight at hatching and early body growth. Two of these varieties, Barred Plymouth Rock and New Hampshire, were known to grow rapidly; while the Oklabar and Legbar varieties had grown slowly in previous years. Data showing the relation between natal testis weight and body weight at eight weeks of age are summarized in Table 1.

TABLE 1

Breed Differences in Natal Testis Weight Compared with Eight-Week Body Weight

		Mean natal testis weight		Mean body weight at 8 weeks
Variety	No.	mg.	No.	lbs.
New Hampshire	38	9.7	40	1.78
Barred P. Rock	28	9.6	221	1.65
Oklabar	120	6.4	112	1.49
Legbar	42	5.3	27	1.28

To minimize the seasonal influence on early growth, males for the growth records were hatched within a two-week period and each brooder contained a representative number of each variety. It will be noted that testis weight at hatching correctly identified the more rapidly growing varieties. Adult body weights for the Barred Plymouth Rock, New Hampshire, and Oklabar are similar. Those for the Legbar are much smaller and it is not known what influence this might have had on the early post-natal growth of this variety.

To further test the feasibility of identifying strain differences in growth rate by testis weight of the baby chicks, 30 dozen hatching eggs were secured from each of two strains of Barred Plymouth Rocks. Average weight of the eggs in the two lots was similar. At hatching 50 male chicks from each strain were chosen at random, killed, and their testes weighed. The remainder of the chicks, both males and females, were brooded under similar conditions to 32 days of age, when body weights were taken. This age was chosen because

Hammond and Bird (1942) have shown that body weight of growing chicks is most variable between four and five weeks after hatching. Testis weight at hatching and body weight at 32 days are compared in Table 2. Strain *A*, which had the smaller testis weight at hatch-

TABLE 2

NATAL TESTIS WEIGHT AND EARLY GROWTH IN TWO STRAINS OF BARRED PLYMOUTH ROCKS

	At hatching		At 32 days after hatching			
	No.	Mean testis weight (mg.)	Mean body weight (gm.)		No. weighed	
			Male	Female	Male	Female
Strain *A*	50	8.9	317	291	64	103
Strain *B*	50	9.4	351	307	74	125

ing, grew more slowly, providing additional evidence that natal testis size may be used as a measure of potential post-natal growth of the body.

Integrating the above results with that of previous research it would appear that earliness of sexual development is definitely related to early growth of the body. Age at laying of first egg for females of the varieties included in Table 1 is youngest for New Hampshires, and increasingly greater for Barred Plymouth Rocks, Oklabars, and Legbars respectively. Earliness of sexual development is, therefore, expressed by a greater natal testis size.

It will be shown later that body size of the newly hatched chick has very little, if any, influence on its testis size. The results of many research workers have proven that chick weight is largely determined by size of the egg from which it hatches. The results presented here indicate that egg size does not affect growth of the testis during embryonic life of the chick. Variations in natal testis weight are probably produced by a variation in the stimulation from the anterior pituitary gland as well as responsiveness of the testis tissue to the gonadotropic hormones. Embryonic testis growth and subsequent body growth must be interrelated through an endocrine balance within the bird's body. The data presented above do not eliminate the influence of adult body size on natal testis weight.

TESTIS WEIGHT AND SIZE OF THE GENITAL EMINENCE

There is considerable variation in size of the genital eminence of

day-old male chicks (Canfield, 1940). Increased growth of the eminence during the early post-natal period has been initiated by intramuscular injections of testosterone diproprionate (Kosin, 1942). Should androgens be secreted by the testis prior to hatching, testis weight might provide an index of their secretory activity. Assuming a similar action of these male hormones prior to hatching, variation in androgen secretion should affect the size of the genital eminence.

To test whether testis weight was associated with size of the genital eminence, 198 day-old White Leghorn male chicks were used. By cloacal examination these were sorted into three groups. There were 23 in the large, 106 in the medium, and 69 in the small eminence groups. Although mean testis weights were 7.0, 6.8, and 6.5 mg. respectively, the difference between these means was statistically non-significant. Size of the genital eminence had no prediction value for testis weight in these day-old chicks.

Testis Weight During Early Post-Natal Growth

After hatching, the body of the chick is freed from its previous growth limitation (egg size) and size to be attained may begin to exert its influence on body growth. In a heterogenous population there are at least two growth stimuli, adult size and sexual development. The exact influence on each is difficult to evaluate. This explains the reason for no linear relation between age at which a certain body weight is attained and average testis weight at that time (Table 3).

TABLE 3

Testis and Comb Weights for Barred Plymouth Rock Cockerels Weighing 250 to 260 gms.

No. of cockerels	Age (days)	Testis-weight (mg.)	Comb weight (mg.)
14	28	47	125
17	29, 30	52	109
16	31	47	103
10	32	48	98
16	33	51	102
26	34, 35, 36	48	81
15	37, 38, 39, 40, 41	64	114

Testis weight is affected by body weight of the chick during its growth. Two methods for removing the influence of body size from the data have been used in previous research. One method consists of

expressing the testis weight as a percentage of body weight. The weakness of this procedure is that the percentage of body weight in testis tissue changes with the growth of the bird. Munro and Kosin (1943) have recently used deviations from the regression of testis on body in their population. Another method of approach was used in collecting the records for Table 3. These Barred Plymouth Rock cockerels were killed as each reached a body weight of 250 to 260 grams. As mentioned previously there was no significant relation between testis weight and age at attaining this specific body weight, except for those few cockerels which were slowest in reaching the 250 to 260 grams. The larger testis weight in these very slow growing males is probably due to thinness of their body. Testis weight may be more nearly related to the proportion of bone and flesh in the body. The greater fatness of the more rapidly growing chicks would decrease the percentage of weight due to flesh and bone.

Inheritance of Natal Testis Weight

Included in Table 4 are the average body and testis weights of 12 newly hatched chicks from each of 46 dams. Those dams mated with sires *A*, *B*, and *C* were in their second laying year. As a result of the larger egg size from two-year-old hens, body weight of their chicks was larger than for first-year hens mated with sires *D*, *E*, and *F*. It may be readily observed that there is no consistent relation between the averages for body and testis weights. The correlation coefficient for the original data was .05, showing testis size to be independent of chick size at the time of hatching.

The differences in mean testis weight between the progeny of sires and between the progeny of dams mated to the same sire are highly significant statistically. Testis weight of the newly hatched chick is, therefore, influenced by both the sire and the dam. The variation between progeny of different dams mated to the same sire emphasizes the hereditary nature of this intraembryonic testis hypertrophy.

The records presented in Table 4 were collected over a period of three months, January through March. Differences between chicks hatched in different hatches from the same dam proved to be statistically non-significant. Whether there might be a seasonal effect on natal testis size at other seasons of the year is not known at the present writing.

TABLE 4

CHICK AND TESTIS WEIGHT IN PROGENY OF INDIVIDUAL DAMS

Sire	Dam	Average for 12 chicks: Chick weight gm.	Testis weight mg.
A	1	46.7	7.3
	2	45.5	5.9
	3	45.8	6.1
	4	50.7	9.2
	5	48.7	6.2
	Sire A Mean		7.0
B	1	48.2	6.8
	2	43.8	5.9
	3	43.2	8.9
	4	42.7	8.4
	Sire B Mean		7.5
C	1	45.2	5.9
	2	48.2	8.0
	3	48.0	6.2
	4	44.4	6.5
	5	45.6	6.6
	Sire C Mean		6.7
D	1	40.1	7.1
	2	37.8	5.7
	3	43.9	6.1
	4	46.4	6.4
	5	38.5	6.6
	6	40.3	6.3
	7	39.6	5.8
	8	44.7	7.5
	9	42.9	6.4
	10	40.3	5.8
	Sire D Mean		6.4
E	1	42.0	5.5
	2	38.4	6.0
	3	40.6	8.8
	4	37.9	6.4
	5	40.5	6.7
	6	40.8	8.0
	7	40.8	7.3
	8	43.0	7.3
	9	43.1	8.1
	10	41.8	8.2
	11	35.7	6.0
	Sire E Mean		7.1

TABLE 2 (*continued*)

Sire	Dam	Average for 12 chicks	
		Chick weight gm.	Testis weight mg.
F	1	38.4	8.3
	2	35.5	9.3
	3	38.5	7.6
	4	38.9	7.1
	5	47.1	8.6
	6	44.0	8.2
	7	44.2	10.6
	8	37.9	8.0
	9	40.5	7.0
	10	41.0	10.5
	11	39.0	7.4
	Sire F Mean		8.4

Further data regarding the heritable nature of natal testis weight was obtained by crossing large and small testis-size lines. Four pens each mated with one male were used for this purpose. Table 5, Group

TABLE 5

INFLUENCE OF CROSSBREEDING ON NATAL TESTIS WEIGHT

	Sire	Dams	No. of progeny	Mean testis weight	
				Observed (mg.)	Expected (mg.)
Group *A*	Legbar	Legbar	42	5.34	——
	B.P.R.*	B.P.R.	28	9.58	——
	B.P.R.	Legbar	35	8.15	7.46
	Legbar	B.P.R.	31	7.50	7.46
Group *B*	Oklabar	Oklabar	116	6.51	——
	N. H.**	N. H.	38	9.66	——
	N. H.	Oklabar	61	8.54	8.08
	Oklabar	N. H.	30	8.48	8.08

*Barred Plymouth Rock.
**New Hampshire.

A, gives the results for the Legbar, Barred Plymouth Rock and their reciprocal crosses. Group B includes similar data for the Oklabar, New Hampshire and their crosses. The average testis weight for the two parental breeds is given as the expected for the crossbreds. In three out of the four crosses the average testis weight was larger than the mean for their parental breeds.

These results from crossing breeds which differed in natal testis size are similar to the results of breed crosses on early post-natal growth which are summarized by Jull (1940). Further tests will be necessary to determine whether superior early growth observed in

certain crosses is associated with a larger natal testis size. According to the conclusions discussed above, early post-natal growth of the body would be expected to be no greater than the average of the parental breeds only in the progeny of the Legbar sire and the Barred Plymouth Rock dams. Should potential growth rate of crossbreds be predictable from natal testis weight, this should constitute a much simpler classification of the value of different strains for crossing to produce poultry meat.

The difference in testis weight between the progeny of reciprocal crosses (Table 5, Group *A*) suggests the possibility of a sex-linked gene affecting natal testis size. It is known that at least one sex-linked as well as autosomal genes affect the age of sexual maturity in females (Warren, 1934). The association previously mentioned between early sexual maturity of females and large natal testis size of their brothers, lends further support to the hypothesis of the action of a sex-linked gene affecting natal testis size.

Summary

Data for this study included 1,573 testis weights and records during the early growth of 880 chickens. Strains of chickens having a larger natal testis weight grew more rapidly in the early post-natal period.

Within the limits of these experiments, natal testis weight was not affected by body weight of the chick or time of hatching. Variations in natal testis size were shown to be highly heritable. Evidence of hybrid vigor and the action of sex-linked as well as autosomal genes on natal testis size was indicated from the records of reciprocal breed crosses. The practical application of selecting for potential growth rate of different strains, the relation to early sexual maturity of females, and the nature of the endocrine basis for variation in natal testis size are briefly discussed. Size variations in the genital eminence of 198 day-old White Leghorn male chicks were probably independent of testis weight.

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THE RÔLE OF HYDROPLASMIC PRESSURE IN HYDROID GROWTH

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(Received for publication on October 2, 1944)

A continuous pictorial sequence of the growth of an Obelia hydranth from bud to senescence was filmed at North Truro by Walp and Hammett during the summer of 1939. The motion picture record reveals rhythmic contractions of the coenosarc throughout the hydrocaulus as well as in the developing zooids. With progressing development, these pulsations increase in intensity and frequency, a phenomenon which might escape notice during ordinary microscopic observation, but in the optical comprehension of which the film serves as a time-condenser. Intervals of 15 seconds were allowed to elapse between successive exposures.

The coenosarc contracts in a peristaltic manner, and each wave of contractions exerts pressure on the liquid contents (hydroplasm) of the gastro-vascular tube. This internal force causes outbulgings at the growing point where the newly secreted perisarc is quite elastic. Hydroplasmic pressure is, therefore, pressure over and above internal resistance of static form to compression from without. The present investigation is concerned with the rôle, if any, of hydroplasmic pressure as a mechanical factor in the morphogenesis of hydranths and of gonangia.

A. MATERIAL AND METHODS

Colonies of the marine hydroid Campanularia flexuosa, gathered from the breakwater at Provincetown, Mass., during the summer of 1943 and in September, 1944, served as experimental material. Only freshly collected healthy colonies relatively free from algal and other epibionts were used.

[1]Aided by a grant from the Women's Auxiliary of the Lankenau Hospital Research Institute.—The Problem was suggested by Dr. F. S. Hammett to whom thanks are due for enthusiastic interest and helpful suggestions. Dr. M. D. Burkenroad's taxonomic advice is gratefully acknowledged.

The following three methods were employed in the elimination of hydroplasmic pressure generated by contractions of the hydrocaulal and pedicular coenosarc: (*a*) cutting of early developmental stages of hydranths and gonangia from the mother-colony; (*b*) cutting of the above stages followed by removal of all perisarc from them; (*c*) complete dissociation of naked hydrocaulus, from which the perisarc had previously been dissected away, into reconstituting fusion masses of coenosarc.

1. *Cutting*

Apical buds at various stages of development were cut as close as possible to their points of origin from the pedicel. It need not be stressed that removal of growing zooids from colonies eliminates or reduces not merely hydroplasmic pressure, but other growth-forwarding and growth-retarding factors as well, so that negative findings with regard to subsequent development could not be ascribed to the absence of the pressure-factor. On the other hand, events which do proceed toward completion after elimination of hydroplasmic pressure, may definitely be considered as independent of this factor.

Intact colonies with buds similar to the cut experimental ones served as controls. Tests and controls were cultured simultaneously under like conditions of environment, particularly with regard to glassware, daily changes of fresh sea water, illumination, and temperature.

2. *Cutting and Removal of Perisarc*

In addition to simple isolation by cutting, a number of hydranth buds were "skinned," i.e., all perisarc was carefully dissected off. This procedure reduces the apical orientation and the unidirectional release of pressure at a well-defined locus of least resistance, such as one would expect to occur in a system where a soft liquid-filled tube (the coenosarc) contracts within a chitinous outer tube (the perisarc), the closed distal end of which is more elastic than the rigid lateral walls. While the lateral attachments of the coenosarc are relatively loose, its terminal proliferating region is always in intimate contact with the newly formed perisarc.

3. *Reconstitution of Hydranths from Dissociated Hydrocaulus*

The method employed in obtaining reconstituting fusion masses

from cell aggregates of completely dissociated skinned hydrocaulus is described in detail elsewhere (Hauschka, 1944). The 80 fusion-masses studied did not exhibit the rhythmic contractions and pulsations of the stem-system from which they were derived, and growth of hydranths from the earliest anlagen to maturity presumably in the absence of hydroplasmic pressure was thus made accessible to analysis.

In all three procedures, the experimental material was kept in small Stender dishes containing 30 cc. of filtered sea water which was changed at least once a day. Some of the experiments were conducted at room-temperature, others—especially those requiring prolonged observation—in a water-bath maintained at 17.2° C. ± 1° C. Glassware and operating needles were boiled in distilled water before use. A record was kept of the progressive form changes during growth by means of camera lucida outline drawings at a magnification of 120x.

The interpretation of events during the course of the various experiments was greatly facilitated through reference to the above mentioned film, hydranth development in Obelia and Campanularia being essentially similar.

B. Results

1. *Isolation of Hydranths*

Immature hydranths isolated from the colony, and consequently not under the influence of hydroplasmic pressure generated in the hydrocaulus and pedicel, proceed toward complete organization, regardless of the developmental stage attained by them prior to cutting. This independence of organizing faculty from the internal pressure factor is summarized in Table 1.

TABLE 1

Hydranth Organization and Emergence in the Absence of Hydroplasmic Pressure from Hydrocaulus and Pedicel

(For terminology of isolated stages see Hammett, 1943.)

Stage isolated	Number cut	Number organized	Number emerged	Per cent organized	Per cent emerged
Bud	30	28	12	93.3	40.0
Half-hydranth (Cone stage)	30	27	18	90.0	60.0
Three-quarter hydranth (Cylinder stage)	30	30	30	100.0	100.0

While continued developmental growth is little or not at all influenced by experimental isolation, the ability of isolated hydranths to emerge through the opercular chitin of the closed hydrotheca seems correlated with developmental age at the time of cutting, as shown in the last column of Table 1. This effect, however, appears conditioned by epithelio-muscular attachments of the hydranth to the basal shelf of the hydrotheca.

Seven of the 30 buds and 5 of the 30 cones listed in Table 1 gave rise to mature apical portions of hydranths with fully formed motile tentacles, functional manubrium and distal region of the stomach (text Figure 1, A-H), but without attachments to the hydrothecal base and, therefore, without the faculty to orient hatching movements or to apply tenticular tonus. Not one of these 12 incomplete hydranths succeeded in emerging.

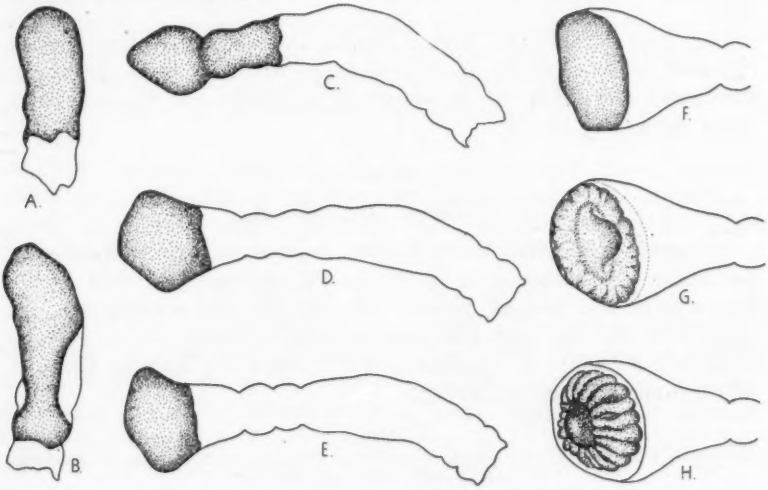

FIG. 1.—ORGANIZATION OF DISTAL PORTION OF HYDRANTH FROM ISOLATED BUD

A. Bud immediately after cutting.
B. After 5 hours.
C. After 20 hours. Note length growth.
D. After 25 hours. Cone stage.
E. After 27 hours.
F. After 45 hours. Cylinder stage.
G. After 49 hours.
H. After 71 hours. Completely organized partial hydranth.

In preparation for emergence, the tentacles of newly formed complete hydranths are extended and pressed rigidly and for prolonged periods against the opercular surface. The tentacles exert such force, that after their retraction longitudinal grooves can be seen in the previously smooth chitinous wall of the hydrotheca.

Whether emergence is entirely dependent on such muscular activity, or whether it is aided by breakdown of the terminal chitin due to enzymes, is not known. The latter possibility is suggested by clusters of dark brown granules egested through the manubrium and adhering to the inner surface of the operculum prior to hatching. It is more likely that these granules are waste products.

Hydroplasmic pressure adds inflation to the independent muscular exertions of the imprisoned hydranth and may be a contributing factor in emergence. Five isolated buds (matched with five control buds attached to colonies) required 64, 60, 58, 57, 56 hours for development and emergence, while the controls emerged after only 30, 28, 27, 24, 24 hours. This considerable rate difference may, however, be ascribed chiefly to differences in nutritional state between isolated and attached zooids (born out by the considerably larger size of the latter), internal pedicular pressure being only a minor accessory factor in emergence.

2. *Isolation of Gonangia*

The gonangia of Campanularia are born on very short stalks, so that contractions of the pedicular coenosarc could generate only insignificant internal pressure with little influence on outgrowth. If pressure is effective at all in gonangial growth, it would have to be supplied by peristalsis in the hydrocaulus and consequent surge of hydroplasm into the hollow blastostyle.

Table 2 summarizes the histories of isolated gonangia, cut from

TABLE 2

GONANGIAL DE-DIFFERENTIATION AFTER ISOLATION FROM HYDROCAULUS

Developmental stage of isolated gonangium	Number cut	Per cent Continued differentiation	Per cent De-differentiation	Per cent Stolon-formation	Per cent Hydranth formation
One-quarter	48	58.3	41.7	29.2	16.6
One-half	42	80.9	10.1	4.7	2.3
Three-quarters	41	97.5	2.5	0	0

their mother colonies with the inclusion of as little pedicular material as possible. The gonangia were isolated at three developmental levels, designated arbitrarily as one-fourth-, one-half-, and three-quarter-stages.

Gonangia which at the time of cutting bear clearly delimited gonophore buds along the blastostyle are capable of continued differentiation. Some of the gonophores continue to grow at the expense of

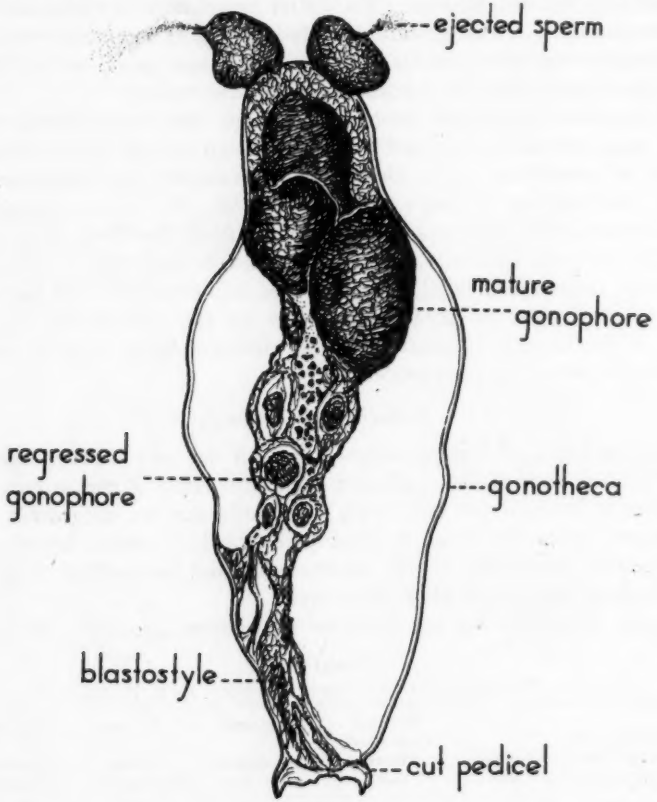

FIGURE 2

MALE GONANGIUM FIVE DAYS AFTER ISOLATION

Continued differentiation of spermatozoa in the older gonophores, while the younger gonophores show regression.

the less advanced ones, the latter being completely resorbed (text Figure 2). This regulation is sometimes followed by degeneration and cytolysis, but in most cases full differentiation of ova, motile spermatozoa, and planulae is attained. The younger isolated gonangia exhibit a relatively high percentage of de-differentiation, the incidence of which is inversely related with developmental age at the time of cutting.

There may also be a correlation between sex and de-differentiation capacity. This is suggested tentatively on the basis of data obtained with male gonangia during September, 1944. Of 93 male gonangia isolated at various developmental levels (including 27 very early buds) none gave rise to hydranths, only one formed a short stolon and 100 per cent produced motile spermatozoa. Of 38 male and female gonangia in approximately equal numbers, cut at three developmental levels during the late summer of 1943, only 24 per cent continued to differentiate, 76 per cent regressed or de-differentiated,

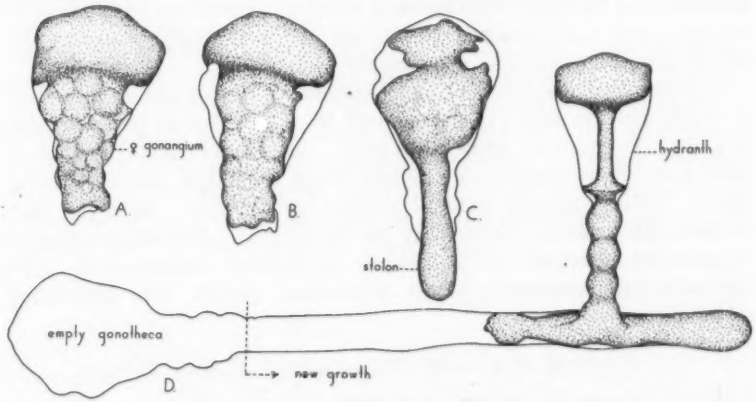

FIGURE 3

DE-DIFFERENTIATION OF ISOLATED FEMALE GONANGIUM AND RE-DIFFERENTIATION INTO STOLON AND HYDRANTH

The sex of a young gonangial bud may be identified by reference to the older gonangia of the same colony, Campanularia being dioecious.

A. Gonangial bud immediately after cutting.
B. After 19 hours. Gonophores are regressing.
C. After 74 hours. Stolon growing from cut end.
D. Ater 140 hours. Gonotheca and part of stolon tube are completely empty. New hydranth in cylinder stage.

39.8 per cent formed stolons, and 23.6 per cent produced hydranths (text Figure 3).

Both the 1943 and 1944 data are included in Table 2. Further work is planned to decide whether the differentiation of female gonosome is actually more reversible than that of male reproductive tissue; or whether there is a fixed limit of indeterminacy in both.

The term "de-differentiation," as employed here, signifies complete regression of blastostyle and of gonadal tissues, frequently followed by stolon-formation and occasionally by re-differentiation into a functional hydranth.

In this trans-substantiation the entire living contents of the gonangium are rebuilt into stolons and hydranths, growing forth from the cut proximal end of the gonangium and leaving an empty, non-living gonotheca (text Figure 3).

The above findings show that gonangial differentiation may continue in the absence of hydroplasmic pressure; they also supply further evidence that hydranth development does not depend on hydroplasmic pressure, but may proceed even from the practically stalkless cut end of a re-differentiating gonangium.

Remolding of the contents of a gonangium takes place in five days at room temperature. It should not be attributed to the activities of a few "embryonic" interstitial cells. More probably, the state of differentiation in coelenterate tissues being reversible and capable of drastic shifts, indicates general cellular totipotency. In these phenomena the pressure factor may be an indirect adjuvant, insofar as it promotes increased circulation of hydroplasm, maintenance of an advanced differentiated state depending partly on circulating metabolites.

3. *Removal of Perisarc from Isolated Hydranth Buds*

After isolation by cutting, a number of hydranth buds were carefully peeled free from the enveloping perisarc. Only 12 came through this delicate dissection without injury.

The "skinned" zooids develop to full maturity much as do intact animals (text Figure 4). Vital staining with Nile-blue sulphate of the proximal end in four of the above 12 individuals showed that the antero-posterior disposition of the various self-differentiating body-regions is unaffected by removal from the perisarc, and that there is

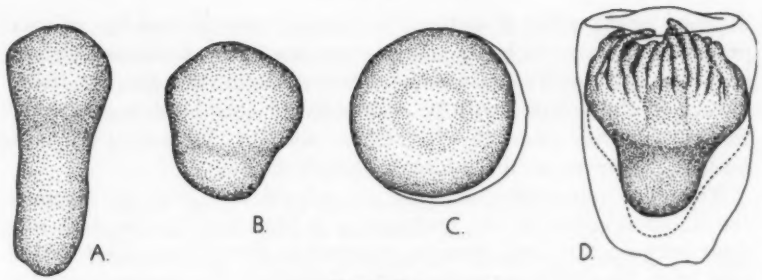

FIGURE 4

DIFFERENTIATION AND ORGANIZATION OF ISOLATED AND "SKINNED" HYDRANTH BUD FROM WHICH THE PERISARC HAS BEEN DISSECTED OFF

A. Naked bud immediately after operation.
B. After 3 hours. Cone stage.
C. After 8 hours. New perisarc has been deposited. The hydranth is viewed from beneath.
D. After 27 hours. Completely organized hydranth, not attached within hydrotheca and therefore unable to emerge. The dotted line indicates change of shape during muscular activity.

also no cell-translocation from cut end toward apex. The originally anterior portion gives rise to tentacles and manubrium, the posterior portion forms the stomach. New perisarc is secreted by naked buds and cones, while naked cylinders cannot deposit a new hydrotheca.

Denuded buds exhibit muscular contractions which were studied with the aid of camera lucida outlines drawn at 30-second intervals and covering the range from total expansion through maximum contraction back to total expansion (text Figure 5).

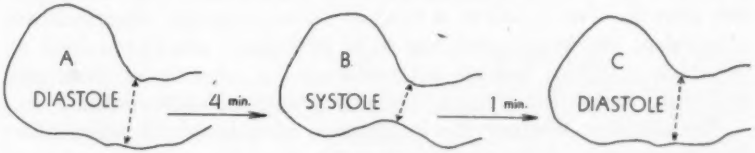

FIGURE 5

MUSCULAR ACTIVITY IN GASTRIC REGION OF DENUDED HYDRANTH BUD

The motility of gastric tissue increases with developmental age, serving as an index of cell specialization and functional differentiation. It should be stressed that the contractility of the stomach walls of the growing hydranth is to be kept distinct from passive expansion

imposed by the influx of hydroplasm pumped into the bud by pedicular peristalsis. Only the latter can be experimentally eliminated through isolation and thereby analyzed as a potential growth adjuvant. Elimination of the former could be accomplished only by drugging which would drastically interfere with cellular activities in general, throwing no light whatever on the internal pressure factor.

That the independent contractions of gastric tissue do compress the liquid contents of the coelenteron is born out by the time relations between the form extremes pictured in test (Figure 5). Contracture from the totally expanded state to minimal diameter requires 3 to 4 minutes, return to maximal diameter only ½ to 1 minute.

This time relationship between contracted and relaxed maxima suggests that the slower systolic phase is under the active control of the epithelio-musculature, which compresses the hydroplasm, while the passive and much briefer diastolic phase involves pressure release during an interval of increased elasticity due to muscular relaxation.

4. *Dissociation of Hydrocaulus Coenosarc*

Naked hydrocaulus coenosarc teased free from its chitinous perisarc-covering and then dissociated into cell blocks not exceeding 150 microns in diameter will fuse into a hemi-spherical mound and secrete new perisarc within a few hours (Hauschka, 1944).

The fusion masses may contain from one to several gastrovascular cavities exhibiting circulation of food particles stirred up by the flagella of the nutritive endothelium; but they do not show rhythmic contractility resulting in an oriented pressure release at regions of new growth. Nevertheless, a total of 80 such masses, observed over a period of 168 hours, gave rise to 88 hydranths and 112 stolons, all of which exhibited normal outgrowth in the absence of peristaltic pulsations emanating from a tubularly organized hydrocaulus.

The question whether the localization of a hydranth anlage, i.e., initiation of hydranth growth, is brought about through internal pressure changes, is likewise answered in the negative by the dissociation method. For hydranth anlagen may appear anywhere along the surface of the fusion mass with no special relationship to the gastrovascular cavities within the mass.

Neither initiation nor subsequent developmental growth, culminating in the fully organized hydranth, depends on hydroplasmic pres-

sure as a morphogenetic factor. It is more in keeping with observed facts to state, that pressure generated by peristalsis is the result of the functional specialization of coenosarc during growth and is, therefore, one of the effects of developmental growth rather than one of its causes.

C. Discussion

Study of the influence of atmospheric pressure changes on life has received a boost through war-enforced aviation medicine.

The effects of hydrostatic pressure on biological phenomena have also been thoroughly investigated. Indicative of recent trends in this field is the work of Johnson, Brown, and Marsland (1941) on narcosis, of Harvey (1942) on stimulation and cyclosis, of Ebbecke and Haubrich (1939) on blood clotting, of Marsland and Brown (1941) on sol-gel equilibria, of Pease (1940, 1941) on cleavage pattern, mitotic spindle, and chromosome movements.

In all these experiments, enormous pressures are needed to produce measurable results. Thus, the cleavage pattern of Chaetopterus shows abnormality only after exposure to 1,400 pounds per square inch (Pease, 1940). Hydrostatic pressure of 1,250 to 3,950 pounds does not delay or prevent normal blood coagulation (Ebbecke and Haubrich, 1939). There is little slowing of cyclosis in the alga Nitella flexilis exposed to pressures up to 6,000 pounds and the effect is reversible, unless the cells have been kept at high pressure too long (Harvey, 1942). At 3,000 pounds per square inch the visible mitotic spindle in Urechis eggs is destroyed, 4,500 pounds very greatly slows movement of chromosomes, 6,000 pounds blocks chromosome movement (Pease, 1941).

The rich fauna brought to light by dredgings from great ocean-depths, where pressure exceeds 3,000 pounds per square inch, suggests adaptability of protoplasm to a wide range of hydrostatic pressures.

In spite of this relative insensitivity to evenly applied pressure, protoplasm is extremely sensitive to uneven pressure, as stressed by Heilbrunn (1943): "A slight pressure exerted on a part of an ameba will stop its motion; if the streaming protoplasm of a plant cell is subjected to mechanical impact, the streaming stops; muscle can be made to contract by mechanical shocks of no great intensity."

Published data on the pressure factor in morphogenesis of cell and tissues are exceedingly scarce. Carey (1921, 1924) raised the quantity

of fluid passing through a dog's bladder from 250 cc. to 50,000 cc. per day (a two-hundred-fold increase). As a result of this increased function, and possibly also increased pressure, the bladder wall thickened from 0.5 mm. to 5.0 mm., and the originally smooth musculature showed striations closely resembling those of cardiac muscle. The bladder wall pulsated rhythmically 200 times a minute. These results suggest that increased function and, more specifically, prolonged internal pressure may modify structural and functional differentiation of tissues.

Selye (1943), experimenting with castrate albino rats, found that "testosterone in itself does not induce full scrotal development in immature rats . . . unless the scrotum is mechanically distended" by the epididymis or an artificial glass testis. Mechanical pressure from within, or distention apparently sensitizes the scrotum to the action of testosterone. "Without this mechanical stimulus the scrota of castrate rats remain subnormal even though the animals receive testosterone in doses sufficient to develop the seminal vesicles and prostates far beyond their normal size."

The rôle of internal muscle-generated hydrostatic pressures as potential mechanical factors in the developmental growth of the organism which generates them has been approached through speculation rather than through experimentation, chiefly because of the technical difficulties of eliminating internal pressures without destroying vital organization, and because of the capillary dimensions of the tubular systems through which these pressures are exerted.

The considerable physiological independence existing between the individual functional members of hydroid colonies makes them suitable subjects for an analysis of the rôle of internal hydrostatic pressure in growth.

The three distinct techniques employed in the present investigation were aimed at: (*a*) elimination of pedicular and hydrocaulal pressure by isolation; (*b*) reduction of internal tension by peeling isolated buds from their rigid perisarc covering, which represents an obstacle to elastic expansion; (*c*) complete disorganization of the pressure-generating system and study of reconstitutional hydranth development.

In spite of their limitations, these methods have made it clear that ***hydroplasmic pressure is dispensable in the developmental growth of a hydranth.***

There is some indication that internal pressure may assist in hydranth *emergence*.

The greater *size* attained by control hydranths (attached to colonies during their development) could be contributed to by *stretching* of the growing bud at each forward surge of hydroplasm during pedicular contraction.

In addition to its stretching effect, hydroplasmic pressure no doubt increases *circulatory efficiency*.

The elasticity of the system within which hydroplasmic pressure is generated and against which it is exerted, combined with the practical incompressibility of the aqueous hydroplasm, makes it appear improbable that the pressure increase is very large in terms of atmospheres. In the absence of actual measurements, possible effects on solubility or osmotic activity are matters of conjecture.

The fluctuating nature of the pressure which, although evenly distributed over the inner surface of the coelenteron, is uneven in time. may further oxygenation and exchange of nutritive and waste substance between adjacent cells and tissues.

Finally, the well-known phenomenon that mitotic spindles tend to orient vertically to the direction of mechanical pressure might be correlated with the distal widening of the hydranth bud into the base of an expanding inverted cone. However, this expansion takes place also in the absence of pedicular pressure.

Contractions of coenosarc in regressing zooids and in stems of Campanularia and Obelia were observed and were compared with similar pulsations in Perophora and other Ascidians by Huxley and de Beer (1923). These authors discuss the possibility that the contraction in a hydroid pedicel "is a mere surface tension effect, exerted passively on relaxation of the walls of the stem." The time relationship shown to exist between systole and diastole in a growing Campanularia hydranth (text Figure 5) makes it appear more likely that contraction is brought about by muscular effort, while return to maximal diameter is the consequence of muscular relaxation.

Huxley and deBeer consider the flow of liquid and of cells from zooids as the cause of "considerable internal tension" to which the cells of the stem react by contraction. In other words: contraction is produced by tension. But what of the tension, i.e., hydroplasmic pressure which is produced by contraction? Assuming that the

epithelio-musculature responds to tension by contracting, would not contraction against the practically incompressible liquid hydroplasm add to the already existing internal tension and thus "act as a stimulus to outgrowth from the stem?"

The great regularity of growth pattern in many hydroids should caution one against ascribing the stimulation of outgrowth to the rather fluctuating internal tension.

On the other hand, there is some evidence of considerable regularity in the contractions of stem musculature. The stalks of Corymorpha, according to Hyman (1940), bend over about 20 times an hour, enabling the hydranths to pick up food particles from the muddy substrate. The stalks will carry on this rhythmic bowing even after decapitation. Rhythm of coenosarc contraction is also apparent in Walp and Hammett's Obelia film.

The observed facts, aside from the experimental data reported above, are: (*a*) Coenosarc is capable of contractions which build up internal tension; (*b*) these contractions are relatively rhythmical.

On the mere basis of these two observations, and the superficial resemblance between a glass unduloid and a Campanularian hydrotheca, D'Arcy Thompson (1943) envisions hydroid growth and form in terms of medusoid splashes of liquid gelatin, adhesive tape stretched over curved surfaces, and soft glass tubing inflated by the breath of the glassblower, who does to the bottle what hydroplasmic pressure supposedly performs in the developing hydranth.

Picturesque analogies of this sort are useful reminders of the potentialities of physical force, viz., surface tension and pressure equilibria, in determining the shape of a living form. But an organism is more than a "diagram of forces" to which protoplasm conforms passively. In spite of "continuous adjustment of internal relations to external relations" protoplasm is basically non-conformist in the sense that it does work and expends energy in the molding and maintenance of differentiated form against external force. In this respect, living matter differs from soap bubbles. The living form does not owe its existence to surface tension, but exists despite limitations imposed by surface tension.

As Hammett (1943) has pointed out, "It must not be forgotten that what the form is to be is determined by genetic direction and

not by internal pressure. Which is to say, the expression and extent of pressure effect is limited by genetic determination."

D. Conclusions

Hydroplasmic pressure, generated by peristaltic contractions of the coenosarc in hydrocaulus and pedicel, is a dispensable factor in differentiation and organization of hydranths, as evident from three experimental procedures, viz., isolation of buds, removal of perisarc, and complete dissociation of hydrocaulus coenosarc, as well as from occasional re-differentiation of the tissues of isolated gonangial buds into complete functional hydranths.

The pressure factor may aid in the emergence of young hydranths through the terminal closure of the hydrotheca. By continued rhythmic stretching of the growing bud and through increased circulatory efficiency internal pressure may assist in the attainment of greater size.

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Growth, 1944, 8, 337-339.

SPONTANEOUS DOUBLE TAILS IN DUGESIA TIGRINA*

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(Received for publication on October 10, 1944)

It is a well-known fact that double-tailed planarians can be produced in the laboratory by experimental cutting. The purpose of this report is to record the observation that they occur in nature also, and at a frequency too high to be accounted for by accidental injury.

The planarians examined were collected in a still water pond located between Wellfleet and Truro, Mass., and were identified as Dugesia tigrina (Hyman, 1939). In the laboratory the animals were kept in large stender dishes in filtered pond water.

The phenomenon of spontaneously occurring double tails was observed in cultures immediately after collection and also in mass cultures of Dugesia kept in the laboratory for a period of up to two weeks. In a culture of 400 animals, double-tailed individuals were observed to total 1.0 per cent immediately after collection. In another fresh collection of 400 worms, three double tails were noted which is an incidence of 0.75 per cent. In collections totalling 1,600 animals from cultures kept in the laboratory for two weeks and fed egg yolk once during that period, 36 individuals exhibiting varying degrees of the double-tailed condition were found (Figures 1-7). This is an incidence of 2.25 per cent.

The types of double tails observed range all the way from a small tip of an accessory tail protruding laterally from the middle of the animal (Figure 1) to two large well-formed tails of equal size (Figures 5, 6, 7). Several of the latter worms had two pharynges (Figure 7) and others only one (Figures 5, 6) depending on the length of the two tails relative to the total body length. The size of the additional tail does not depend on the size of the animal. Both large and small animals were observed to have either large or small accessory tails (Figures 3, 5, 6).

The double tail was cut from one animal which regenerated a single

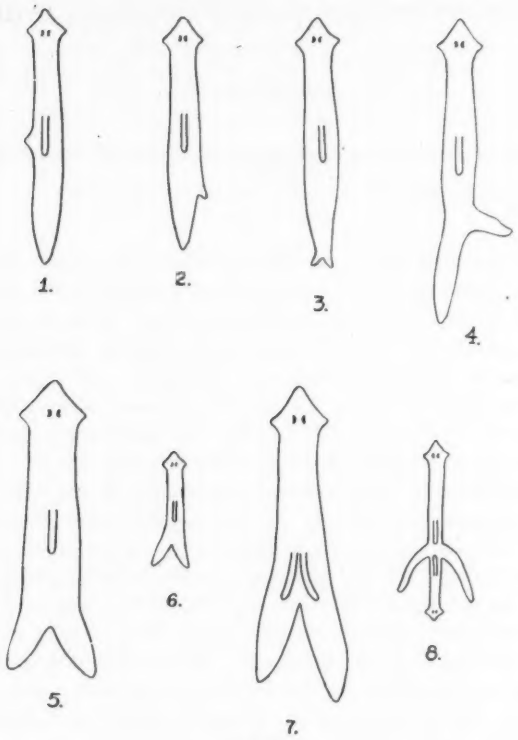

CHART 1

Figures 1-7. Various degrees of the double-tailed conditions in Dugesia tigrina (x 7). Figure 8. A bipolar specimen with two heads at opposite ends and two tails protruding laterally (x 7).

tail at the cut end, while the removed double tail gave rise to a very small head within four days.

When fission takes place in double-tailed animals, the whole double tail is pinched off if there is a single pharynx. If two pharynges are present each tail is pinched off separately. This suggests that the double-tailed condition is probably not the result of suppressed fission.

Bardeen (1903) has reported spontaneously occurring freaks removed from the egg capsules of Planaria maculata and concludes that

"irregularly formed embryos are not uncommon." Stockard (1921) has reported double monsters in trout eggs, Fundulus, and chick eggs which he attributes to the retardation of the developmental rate of the embryo at some critical point. It is of interest that spontaneous abnormalities of the head region occur very rarely since in all the collections recorded above, only one head anomaly (as against 43 tail anomalies) was observed. This was a bipolar animal which had two heads at opposite ends and two tails protruding laterally (Figure 8). The embryonic freaks which Bardeen describes are also largely animals with double tails and multiple pharynges, and include only one twin specimen. Monti (1900) has described various adult freaks collected in Alpine streams. She attributes them to injuries caused by being dashed against rocks by rapidly flowing water. The animals recorded here were collected from a small still water pond well protected from winds so that selective injury of the tail by wave action is out of the question. Injury of the posterior end inflicted by a natural enemy such as an aquatic insect or small fish is also unlikely to be restricted to the posterior end only and is probably not responsible for the phenomenon. It is more feasible that the abnormalities are produced in the egg capsule as Bardeen reports.

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*Aided by a grant in memory of Susanna E. Dercum.

Growth, 1944, **8**, 341-344.

BOOK REVIEW

(Franz Schrader. Mitosis: The Movements of Chromosomes in Cell Division. New York: Columbia Univ. Press, 1944. Pp. 110.)

REVIEWED BY FREDERICK S. HAMMETT

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In reviewing this treatise I am torn between admiration and disappointment. Admiration for the manner in which the subject is presented. Disappointment that more of available pertinent data necessary for a well-rounded presentation was not given. In view of the title, the author, the editorial sponsors, the publisher, and the avowed purpose of these volumes, it would seem as if the reader had a right to expect a more adequate coverage of the field than is here given. What he gets for his two dollars is not a book or a monograph on mitosis; but a very fine write-up of the author's predilection to a bio-physical analysis of the movements of chromosomes in cell division. These 86 pages of text and 15 figures, plus 18 pages of references and 6 of faulty indexing would seem to comprise an article more suitable for publication in the *Annual Review of Biology*, and later issue as a pamphlet, than for publication as a book.

I would be the last one to deny any author the privilege of emphasizing in any of his writings those aspects of the subject to which he is particularly attracted. But I do feel that this does not excuse him from including in a monograph intended for general distribution a general survey of all chief lines of inquiry pertinent to his subject. By failing to do this he tends to over-weight the argument in one direction, and to leave the lesser informed reader with the idea that there is no other valid approach.

To be specific—those interested in mitosis—which is the cover title of this book—will find here no reference to the chromosomes of malignant cells as studied by Shields Warren and others. No note on mitosis in amoebae as elucidated by Chalkley and his co-workers. No comment on the effects of colchicine and X-rays. Surely Gurwitsch's

mitogenetic rays could have been given some attention as long as magnetism was not considered too esoteric for extended comment! And to have included Caspersson's work on the chemical composition of the chromosomes would have been fitting to a discussion of factors concerned in chromosome movements in cell division. Finally, one may question a judgment which brusquely brushes aside all factors which may induce mitosis,—and by a reference to Gray's 1931 review, gives the uninformed reader the idea, not only that these are unimportant to chromosome movement; but also that no work worthy of note has been done in this field for the past 13 years!

In his introduction, Professor Schrader admits that cytologists have failed to solve most of the problems of mitosis. In his conclusion he sadly agrees these can't be solved by invoking the bio-physical genii of "—contraction, expansion, hydration, diffusion and electrical forces—." And he is dubious about the genetical approach. And there to all intents and purposes he comes to a frustrated stop.

It looks as if the cytologists have run out of gas. Some kindly soul should give them a lift to the nearest filling station. A filling station where they can get some of the high-test, anti-knock fuel which their somewhat antiquated motor has need of. This fuel has two principles, the consequences of which are guaranteed to revitalize the most recalcitrant mechanism.

The first principle is:—*There are no boundaries in science; there are only interdependencies.*

The second principle is:—There are four principal aspects of things, viz. Direction—which is comprised for Biology in the science of Genetics: Substance—which is comprised in the science of Chemistry: State of Substance—which is comprised in the science of Physics: and Form—which is comprised in the science of Anatomy, of which Cytology is a part. All exist together, indissoluble and ineluctably interdependent.

Mitosis, like every other activity, is compounded of these four basic elements.

The cytological (form) changes of mitosis express a sequence, each stage of which evolves from the preceding, and is itself the origin of the next. These changes are interdependent as Professor Schrader himself attests when he writes: "—it is almost impossible to affect a given structure or process—without simultaneously affecting several others."

The physical (state of substance) changes of mitosis express a sequence, each stage of which evolves from the preceding, and is itself the origin of the next. Here too there is interdependence. Not only between stages; but also between form and the state of the substance of which form is composed. For form is conditioned by the state of its substance: and the state of substance is factored by form.

All the activities of living are subtended by chemical reactions. They are, in effect, the products of these reactions however they may be expressed. Whether by change in form, or change in physical state, or whatever.

Mitosis then is expression of a sequence of chemical reactions or substance change and exchange. And each reaction is the product of the preceding and the point of departure for the next. Here too is interdependence. Not only between the reactions comprising the succession; but also between the reacting substance, the states of that substance, and the forms that substance takes. For state of substance is the product, not the producer of substance; while the rate of chemical reaction is factored by state of substance. And the form substance may take is conditioned by the chemical nature of the substance: while form may factor the extent to which chemical reactions may be expressed.

And the whole is of course set by Direction (Genetics if you will) which determines the cyto-specific kind of substance undergoing mitosis. Which in turn sets the reactions resulting in cyto-specific forms and physical states. Interdependence is not lacking. For the expression of direction is indubitably conditioned by chemical composition and its reaction sequalae.

It is thus evident that mitosis is not cytology, not genetics, not physics; but all these plus chemistry acting interdependently. Such being the case it is no wonder cytologists have failed to promulgate a satisfactory explanation of mitotic phenomena. For, if Professor Schrader's pamphlet may be taken as a guide, the cytologists have sedulously eschewed admitting the chemical factor (nature and reactions of substance) into their hypotheses. And clearly no explanation of any interdependency is possible without use of all the elements of which it is compounded.

We chemists who peck away at problems of growth, development, and mitosis can't get along without data supplied by cytologists, bio-

physicists, and geneticists. And I don't believe the cytologists, geneticists, and bio-physicists can get very far without help from the chemists.

I'll bet a dime to a ginger-snap that the chemists know more of cytology, genetics, and bio-physics, than the practitioners of these disciplines know of chemistry. If this disparity were rectified grief over past failures and future prospects like that keened by Professor Schrader might be mitigated. Two steps could be taken. (*a*) Reorient collegiate instruction in science subjects so that biology students can follow the syncytial connections between them. And thus apperceive that there are no boundaries in science; only interdependencies. (*b*) Establish a required course in bio-chemistry in every biology department. Equal in coverage and intensity to that given in medical schools. Thus turning out graduates completely rather than partially equipped to go on from where we leave off. It could happen that "—the stone that the builders disallowed, the same is made the head of the corner."

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